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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

MORPHINE MEDICATION AS A METHOD OF DELAYING ASPHYXIATION*

BY FELIX P. CHILLINGWORTH, M.D., AND ATTILIO CANZANELLI, M.D., BOSTON

THIS investigation was undertaken in the belief that it might prove of value as a palliative measure to be used in future conditions, such as existed on the submarine S-4, and thereby prolong life.

Under normal atmospheric conditions, an increased need for oxygen goes hand in hand with an increased production of CO_2 which results in stimulation of respiration due to the small temporary excess of CO_2 tension in the blood. On the other hand, when the subject is in an air-tight compartment, this same delicate mechanism hastens asphyxiation.

It is common knowledge that morphine decreases mental and physical functional activity; and it was reasoned that the accompanying slowing of the respiratory apparatus would limit or retard the need for oxygen and decrease the output of CO_2, hence, other things being equal, the subject would live longer because the available oxygen would be utilized more slowly.

As this research is concerned with the reactions of the respiratory center to oxygen and carbon dioxide under mild morphine dosage, a brief review of the behavior of this center as influenced by morphine is given. Dixon[1] states that "small doses of morphine depress the respiratory center in the medulla. The respiration becomes slower, and the inspiration is somewhat prolonged so that it is deeper than usual; the sum total of these two factors is to diminish the respiratory exchange. In a resting rabbit the air expired in thirty seconds averages about 200 c.c. If 0.01 gram morphine hydrochloride is administered as an injection the average soon falls to about 90 c.c., and in man a similar condition obtains, though not so exaggerated as in the example given. In

*From the Department of Physiology, Tufts Medical School.
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larger doses the respiration becomes very shallow, and before death, frequently assumes the Cheyne-Stokes type."

Sollmann[2] states that "Morphine and its derivatives depress the excitability of the respiratory center. The spontaneous rhythm and the response to asphyxia and to reflexes are all lowered."

Y. Henderson[3] points out "that very minute doses (2 mg. of morphine for man) lessen the cough reflexes without producing any other noticeable change. Somewhat larger, but still nonnarcotic doses (3 to 15 milligrams of morphine) diminish the excitability for carbon dioxide, and to a lesser degree for other afferent impulses."

Higgins and Means[4] confirmed the fact that the threshold for CO_2 is raised.

Recently, Y. Henderson[5] in discussing "oxygen want" wrote as follows: "Oxygen is not a stimulant but a foodstuff. Deficiency of oxygen, beyond the first slight stimulating effect, depresses the nerve centers. In the absence of oxygen the tissues of the body cannot produce carbon dioxide. It is the carbon dioxide carried by the blood from the tissues to the brain that is the physiologic stimulant to respiration. When the center is depressed, it requires more than the normal amount of this stimulant to induce activity."

Further data are supplied by Meyer:[6] "Under its influence the respiratory center becomes less excitable, so that the CO_2 in the inspired air must be increased much more than under normal conditions in order to cause the usual increase in respiration. The sensibility of the respiratory center to reflex stimulation, such, for example, as that resulting from stimulation of the sciatic, is diminished in the same fashion as its sensibility to CO_2.

"In man, after very small doses of morphine (3 to 10 mg.) the diminished excitability of the respiratory center expresses itself by slowed and deepened breathing, for a stronger summation of the stimuli (distention of the lungs and the CO_2 tension in the blood) is needed to excite the rhythmic respiratory movements."

While there is decided uniformity of opinion regarding the behavior of the respiratory center to oxygen and carbon dioxide, the reported results regarding the basal metabolic rate under morphine medication are confusing and conflicting. Dixon[7] states, "We have seen already that the absorption of oxygen is considerably diminished after morphine; the carbonic acid output is diminished also, although not to the same relative extent as the oxygen. As a result, the amount of carbonic acid present in the blood is usually increased slightly. Metabolism is lessened, no doubt, on account of the general quiescence. In consequence of these combined effects, less nitrogen is excreted in the urine."

In agreement with Dixon, Sollmann[8] writes that "The output of CO_2 and to a lesser degree of urinary nitrogen, is materially decreased, mainly by the muscular quiet; in prolonged experiments also by the digestive derangement."

Nishigishi[9] contradicts the above authors while Boothby and Rowntree[10] conclude that morphine in sufficient quantities to produce sleep lowers the heat production." It should be borne in mind that the experiments

cited, pertaining to the action of morphine on the basal metabolic rate, were carried out under standard atmospheric conditions. On the other hand, subjects in air-tight compartments are confronted with an atmosphere which is constantly changing; the oxygen percentage in the air of the compartment is progressively diminished whereas the CO_2 progressively increases.

The initial effects of very small amounts of morphine are to lessen the sensibility to pain, cough, hunger, discomforts, and other disturbing influences. Before considering the use of morphine to delay asphyxia in man, tests should be made in order to secure subjects that give normal reactions to this alkaloid.

METHODS OF INVESTIGATION

Preliminary tests were carried out upon white rats in air-tight compartments to determine dosage and differences in time of death between the morphinized animals and the controls.

The initial dose of morphine was determined by using 25 per cent of the textbook dose for white rats which is given as (minimum fatal dose, 0.40 grams per kilo or 0.40 mg./gm.), i.e., 0.10 mg./gm. With this amount, rats invariably died before the controls in the same containers. Next, a further reduction of 50 per cent (0.05 mg./gm.) was tried with similar results.

Realizing that the dose was still too great it was again cut to 0.025 mg./gm. Even with this marked reduction the morphinized rats still died before the controls. This led us to appreciate that we were dealing with a complex depression of the respiratory center, caused by morphine, "oxygen want" plus CO_2 action. From this point we ran our controls in separate containers of equal volume in order to secure a direct comparison, because in the same containers the normal rats were utilizing the oxygen at a more rapid rate, and this utilization naturally added to the depression of the morphinized rats and complicated the analysis of results.

We later changed from museum specimen jars to a bell jar* with two taps, one on the top and one on the side, in order that the gas analyses (by Haldane method) could be carried out. In addition an equalizing manometer was provided which permitted the air-tight jar to have atmospheric pressure at all times. This manometer bulb had a cubic capacity practically equivalent to the respiratory deficiency produced by the rats under observation, and therefore this bulb volume was displaced into the bell jar toward the end of the experiment.

DESCRIPTION OF EXPERIMENTS AND DISCUSSION OF RESULTS

Table I gives in detail the time of asphyxiation of rats both with and without morphine. It brings out in addition several interesting facts pertaining to "oxygen want" and the corresponding reaction of the respiratory center.

Since in our experiments we have the time of asphyxiation of rats of different weights, we must interpolate this weight factor in terms of a standard weight rat. We decided to use a 150 gram rat as our standard unit throughout this investigation.

*Sealed with paraffin.

TABLE I

EXP. 1. 2/15/28		EXP. 2. 2/16/28		EXP. 3. 2/16/28		EXP. 4. 2/27/28		EXP. 5. 2/24/28		EXP. 6. 2/27/28	
Container: Museum jar—4.5 liters		Container: Museum jar—2 liters		Container: Museum jars—1 liter		Container: Museum jars—1 liter		Container: Museum jars—1 liter		Container: Museum jars—4.5 liters	
Dosage: 0.10 mg./gm.		Dosage: 0.05 mg./gm.		Dosage: 0.025 mg./gm.		Dosage: 0.025 mg./gm.		Dosage: 0.025 mg./gm.		Dosage: 0.025 mg./gm.	
No. I Morphine Wt. 130 gm.	No. II Without Wt. 175 gm.	No. I Morphine Wt. 150 gm.	No. II Without Wt. 147 gm.	No. I Morphine Wt. 166 gm.	No. II Without Wt. 142 gm.	No. I Morphine Wt. 140 gm.	No. II Without Wt. 183 gm.	No. I Morphine Wt. 167 gm.	No. II Without Wt. 165 gm	No. I Morphine Wt. 174 gm.	No. II Without Wt. 131 gm.
Time		*Time*		*Time*		*Time*		*Time*		*Time*	
1.45 Morphine		3.00 Morphine		4.28 Morphine		2.53 Morphine		12.28 Morphine		10.08 Morphine	
1.50 Jar sealed		3.04 Jar sealed		4.29 Jars sealed		2.54 Jars sealed		12.30 Jars sealed		10.09 Jars sealed	
2.00 Resp. 200		3.05	Resp. 100	4.30 Resp. 200	Resp. 120	2.55 Resp. 140	Resp. 140	12.40 Resp. 116	Resp. 150	10.12 Resp. 80	Resp. 100
2.30 Resp. 110	Resp. 160	3.30 Resp. 150	Resp. 120	4.40 Resp. 160	Resp. 120	3.15 Resp. 140	Resp. 165	12.55 Resp. 130	Resp. 140	10.15 Resp. 100	Resp. 100
3.00 Resp. 60	Resp. 170	3.45 Resp. 130	Resp. 104	4.50 Resp. 100	Resp. 114	3.29	Resp. 28	1.08 Resp. 100	Resp. 57	12.00 Resp. 50	Resp. 160
3.30 Died	Resp. 120	4.00 Resp. 48	Resp. 80	5.00 Resp. 80	Resp. 108	3.30	Removed	1.10	Resp. 10	12.45 Resp. 50	Resp. 108
4.25	Died	4.10 Died		5.05 Resp. 60	Resp. 36		and resuscitation	1.11 Resp. 80	Died	1.15 Resp. 80	Resp. 108
		4.14	Resp. 36	5.08 Resp. 20	Died		attempted	1.17 Resp. 128		1.25 Died	Resp. 104
		4.20	Died	5.13 Resp. 12			Died	1.20 Resp. 80		1.45	Resp. 100
				Rat removed and resuscitated		3.33 Resp. 108		1.22 Resp. 10		2.10	Died
						3.37 Resp. 80		1.23 Died			
						3.40 Resp. 72					
						3.45 Resp. 40					
						Removed and resuscitated					

This reduction of time values can be made since the time is proportional to oxygen consumption while the oxygen consumption is approximately inversely proportional to the body weight,* thus permitting direct comparisons as shown by evaluations in Table II.

TABLE II

COMPARES DURATION OF LIFE WITH MORPHINE AND WITHOUT MORPHINE IN TERMS OF 150-GRAM RAT

EXP.	DOSE	WITH MORPHINE	WITHOUT MORPHINE
1	0.10	87 min.	181 min.
2	0.05	66 min.	74 min.
3	0.025	49 min.	37 min.
4	0.025	48 min.	44 min.
5	0.025	59 min.	45 min.
6	0.025	228 min.	210 min.

Experiment 1 was the first in which morphine was used. Two rats were placed in a four and one-half liter jar. The morphinized rat lasted eighty-seven minutes while the control lived one hundred eighty-one minutes; here the morphine failed to delay death. This experiment indicated that we were dealing with an early death due to too great a depression of the respiratory center by morphine. It is obvious that the slowing of the use of the available oxygen carried the control a longer time.

Experiment 2 was run in the same container, but its volume was reduced one-half with acidulated water and the rats were supported on a floor above the water level. Here the morphinized rat lasted sixty-six minutes while the control lived seventy-four minutes. The dose of morphine which had been reduced 50 per cent still gave evidence of overdosage. At this point we appreciated that we were dealing with a complex depression of the respiratory center, i.e., from morphine, from "oxygen want," and from carbon dioxide. This prompted us to carry on experiments in separate air-tight containers because it was evident that the rat with the raised threshold would die first.

Experiments 3, 4 and 5 are typical of the effects of morphine upon rats in separate containers (1 liter jars). Here the morphinized rats were saving oxygen for themselves alone, with the result that they outlived the controls. Incidentally, in two cases the morphinized rats were removed and resuscitated. Our usual procedure, however, was to obtain data regarding the exact extension of life with morphine. In Experiment 3 the rat with morphine lasted forty-nine minutes while the control lasted thirty-seven minutes. In Experiment 4 the morphinized rat lasted forty-eight minutes whereas the control lived forty-four minutes. In Experiment 5 the morphinized rat lasted fifty-nine minutes, the control forty-five minutes.

Experiment 6 is a similar test, but in a 4.5 liter container. In this experiment it appears that the morphinized rat died first; however, reducing the time factors in terms of 150 gram rat, we find that actually the morphinized rat lasted 228 minutes while the control lasted 210 minutes.

*If the rat weighed 130 grams and it lived ninety minutes its corrected duration of time would be $\frac{130}{150}$ times 90 which equals seventy-eight minutes.

Experiment 7 (Table III) is a comparison between two groups of rats. In one jar were placed three rats with morphine and one without. In the second jar were placed four rats without morphine. In this experiment all the morphinized rats lived longer than those in the second jar, but the rat without morphine in the morphine group outlived all other rats. This rat was removed and quickly returned to normal.

TABLE III

EXP. 7. 3/5/28	EXP. 8. 3/7/28	EXP. 9. 3/8/28	EXP. 10. 3/14/28
Container: Museum jars—4.5 liters Dosage: 0.025 mg./gm. No. I Morphine 4 rats* Wt. 533 gm. No. II Without 4 rats Wt. 596 gm. *Time* 12.51 Morphine 1.00 Jars sealed 1.42 4 dead 1.45 One dead 1.50 Three dead 1.50 Remaining rat* removed and resuscitated	Container: Bell jar—10 liters Dosage: 0.025 mg./gm. 5 rats* Wt. 724 gm. *Time* 12.52 Morphine 12.57 Jar sealed 2.00 One rat dead 2.17 Two rats dead 2.28 Three rats dead 2.30 Four rats dead Sample taken CO_2 13.94 O_2 4.9 4.23 Fifth rat dead	Container: Bell jar—10 liters 5 rats Wt. 642 gm. *Time* 2.50 Jar sealed 4.12 One rat dead 4.23 Two rats dead 4.24 Three rats dead 4.42 Four rats dead 4.47 Fifth rat dead Sample taken CO_2 14.26 O_2 2.87	Container: Bell jar—10 liters 5 rats Wt. 680 gm. *Time* 1.20 Jar sealed 2.17 One rat dead 2.45 Two rats dead 2.58 Three rats dead 3.00 Four rats dead 3.02 Five rats dead Sample taken CO_2 15.49 O_2 3.84

*One rat without morphine.

TABLE III—CONT'D

EXP. 11. 3/24/28	EXP. 12. 3/26/28	EXP. 13. 3/16/28	EXP. 14. 3/27/28
Container: Bell jar—10 liters Dosage: 0.025 gm./mg. 5 rats Wt. 832 gm. *Time* 11.42 Morphine 11.46 Jar sealed 12.30 One rat dead 12.31 Two rats dead 12.55 Three rats dead 1.00 Four rats dead Sample taken CO_2 11.47 O_2 6.33 2.17 Five rats dead* Sample taken CO_2 12.34 O_2 5.58	Container: Bell jar—10 liters 5 rats Wt. 1126 gm. *Time* 11.45 Jar sealed 12.40 All rats dead Sample taken CO_2 14.21 O_2 4.25	Container: Bell jar—10 liters 2 rats Wt. 282 gm. *Time* 12.28 Jar sealed 5.25 Two rats dead Sample taken CO_2 14.93 O_2 2.58	Container: Stoppered jars—1.5 liters Dosage: 0.025 gm./mg. No. I Morphine Wt. 237 gm. No. II Without Wt. 137 gm. *Time* 12.23 Morphine 12.34 Jar sealed 1.17 Dead Samp. taken CO_2 12.56 O_2 6.06 1.33 Dead Samp. taken CO_2 13.27 O_2 4.34

*One rat without morphine.

Experiments 8, 9, 10, 11, and 12 were carried out in a bell jar to permit gas analysis. Five rats were placed in the bell jar at a time. In Experiments 8 and 11, four of the five rats received morphine. Samples of the air were taken immediately after all the morphine rats had died and the final samples were taken at the end of the experiments. These experiments again show that where the morphine rats and the control rats are together, the control rats live the longest.

Experiments 9, 10, and 12 are groupings of five rats without morphine. Here samples were taken at the end of the experiments.

In Experiment 13, two rats were placed in a bell jar without morphine, samples of air being taken at the end of the experiment. In 14 we utilized a smaller jar to shorten the time of the experiments on account of the reduced number of rats.

TABLE IV

ANALYSES OF AIR IN CONTAINER AT TIME OF DEATH OF RATS

	MORPHINE		WITHOUT MORPHINE	
	PER CENT CO_2	PER CENT O_2	PER CENT CO_2	PER CENT O_2
Exp. 8	13.94	4.90	16.31	3.31
Exp. 9			14.26	2.87
Exp. 10			15.49	3.87
Exp. 11	11.47	6.33	12.34	5.58
Exp. 12			14.21	4.25
Exp. 13			14.93	2.58
Exp. 14	12.56	6.06	13.27	4.34
Average	12.65	5.76	14.40	3.82

Table IV gives the results of gas analyses. In the group studies rats did not die at the same time indicating that there are individual variations in their reactions to anoxemia. This was true regardless of whether they had or had not received morphine.

This series shows in all instances that the morphinized rats had a raised oxygen threshold which gives a distinct explanation for their earlier deaths when in the container with the control. It is also evident that in the death resulting at the higher oxygen level there must necessarily be a lowered CO_2 value. The average CO_2 and O_2 percentage of the air in the container of the morphinized rats was 12.65 and 5.76 respectively, while the percentage of CO_2 and O_2 of the control averaged 14.40 and 3.82 respectively.

The action of morphine is a depressant upon the respiratory center, and upon all activity. This general depression slows respiration in three ways: (1) it reduces the oxygen consumption; (2) at the same time the morphine raises the oxygen threshold; and also (3) raises the CO_2 threshold. One might expect the morphinized rat to die first because its respiratory center for oxygen is set at a higher level, hence, oxygen percentage below this point would be incompatible with its life, but the slowed respiration of the morphinized rat results in a slowed utilization of available oxygen, hence, the oxygen level at which death takes place is reached later than that of the control rat.

Larger dosage produces a heavy depression of the respiratory center, and the oxygen level at which death occurs is soon reached because the slowed respiratory rate does not sufficiently compensate for the raised oxygen level at which life will be maintained, i.e., the rise in oxygen level is not directly proportional to the decreased respiratory rate.

It is possible that the dosage could be further regulated so that the ratio between the decreased respiratory rate and the raised oxygen level would result in the maximum prolongation of life.

That life should be sustained in a modified way at a low percentage of oxygen (about 3 per cent) which occurs before complete asphyxiation is rather startling, yet Haldane was fairly comfortable for a short time with less than 9 per cent, and in gas-oxygen anesthesia, it is customary to allow the patient less than this amount.

All morphinized rats showed that the oxygen threshold was higher than that of the controls. This fact leads us to believe that in cases with marked anoxemia (pneumonia), it would be well to consider using other drugs to diminish the acuity of sensations and to slow respiration, or at least to prescribe morphine in reduced doses with perhaps more frequent administration.

In the above experiments the cubic capacity of the air-tight container in comparison with the volume of the rat was small. It is obvious that increase in container capacity would delay fatal apnea and extend life; the more slowly asphyxiation is produced, the less noticeable are the sensory and motor symptoms.

CONCLUSIONS

1. Data are presented showing that with proper dosage, morphine will prolong life in air-tight compartments.

2. Definite evidence is given that the respiratory center is depressed by a deficiency of oxygen, in spite of increasing carbon dioxide tension.

3. With marked anoxemia very small amounts of morphine affect the oxygen threshold of the respiratory center.

4. Decreased functional activity expresses a slowing of the basal metabolic rate.

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THE ATTITUDE OF THE PROGRESSIVE PHYSICIAN TOWARD NECROPSIES*

By Ralph G. Mills,† M.D., Rochester, Minnesota

I WISH to turn back the pages of history and quote the writings of a man who was much criticized by his fellow physicians for his advanced ideas, and to make his words the basis of my remarks. A physician of Mantua, in Lombardy, named Marcellus Donatus, in 1586 wrote as follows: "Let those who interdict the opening of bodies well understand their errors. When the cause of disease is obscure, in opposing the dissection of a corpse which must soon become the food of worms, they do no good to the inanimate mass, and they cause a grave damage to the rest of mankind, for they prevent the physicians from acquiring a knowledge which may afford the means of great relief, eventually, to individuals attacked with a similar disease. No less blame is applicable to those delicate physicians, who, from laziness or repugnance, love better to remain in the darkness of ignorance, than to scrutinize, laboriously, the truth, not reflecting that by such conduct they render themselves culpable toward God, toward themselves, and toward society at large."

It is the latter part of this quotation to which I would direct your attention. Scholarship alone does not necessarily imply conviction that necropsies should be conducted after every fatality. It is a fundamental weakness in our medical educational system that allows students to be graduated without being so fully convinced of the supreme value of necropsies that they would unhesitatingly grant permission for its performance on themselves or a member of their family. I am assuming, of course, that the procedure would be conducted in a satisfactory and deferential manner, in keeping with the solemnity of the occasion and with the importance of the examination to all concerned.

Much of the medical knowledge available today has come directly from the necropsy table. Even a cursory survey of medical history will convince the skeptic of this fact. The Alexandrian school flourishing about 300 years before the Christian era was exceedingly active in the study of pathologic anatomy. The zeal of these physicians would put most of us to shame, but unfortunately with the fall of their civilization their medicine went with it. The Roman and later civilizations that supplanted it failed among other things to make use of the examination of dead bodies, but allowed the mind and imagination full sway. The result you know only too well. The Dark Ages were dark indeed for medicine, and it was many centuries before the light began to appear.

*Abridgment of address presented at the Alpha Omega Alpha Banquet, Omaha, Nebraska, January 20, 1928.

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†From the Section on Pathologic Anatomy, Mayo Clinic, Rochester.

It is significant that the real beginnings were made by men living in the Roman States, which were later united into what we know today as Italy. A group of four men, Anthony Benivieni, then Eustachius, Rembert Dodoens, and finally Marcellus Donatus, were outstanding. It is recorded, however, that in spite of their enthusiasm and earnestness pathology did not make very rapid progress. Opposition was too strong and there were no aggressive physicians to carry on the work so energetically inaugurated.

A beginning was being made in England about this time. Francis Bacon urged that necropsies be performed for the advancement of medical knowledge. William Harvey and Glisson were of the faithful few who made practical use of necropsy material. (Glisson, too, was an ardent advocate of finding the lesions that accompany a certain train of symptoms.)

Morgagni, another Italian, nearly 200 years later, should be credited with pushing aside some of the gloom that had covered medical science for so many centuries. He it was, in 1761, who published a work, "The Seats and Causes of Diseases Investigated by Anatomy," that marks the beginning of a new era. Only since his time have necropsies become so frequent as noticeably to affect medicine.

A semblance of accurate diagnosis came into being when the performance of necropsies was more of a routine than an event. Laennec, in France, was able to interpret breath and heart sounds in terms of pathologic anatomy by means of the stethoscope which he invented, and with constant study of his patients after death. Bright made his contribution to nephritis in a similar manner. Would that there were more of his type today to complete the work so nobly begun. Semmelweiss, in Austria-Hungary, was impressed by the similarity between the organs of those dead from puerperal fever and of those dead from septicemia. "Infection was his conclusion and cleanliness his remedy." Fitz, an American, discovered appendicitis at necropsy.

It is significant that practically all the men of note whose names you can recall as being outstanding in the medical practice of the past have had one thing in common. They have been clinicians who followed their fatalities to the necropsy table. The mention of the names of a few of these will suffice: Hunter and his nephew, Baille, Bichat, Graves, Corrigan, Skoda, Hodgkin, Stokes, Delafield, Janeway, Halsted and Osler. Thus has the profession profited by the experience of these masters of clinical medicine who were at the same time experts in the interpretation of pathologic conditions. Many of the great advances of the past have been made as a direct result of the intensive study of pathology as revealed at the necropsy table.

You are doubtless familiar with the biologic dictum that ontogeny recapitulates phylogeny. I have already shown that the science of medicine has been directed and moulded by the correlation of the clinical and pathologic phenomena. I wish to point out the manner in which the individual grows, and to develop the thesis that he is subject to exactly the same principles as apply to the evolution of the science.

The best diagnosticians of this and past generations have been those most careful to correlate clinical with pathologic data. The example of Osler will

be remembered in this connection, by his oft-repeated remark that he never missed a necropsy in one of his fatal cases. This constituted a previous engagement, that took precedence over every other appointment no matter how important, and regardless of the prominence of the individuals concerned. Continental physicians have been more exacting in this particular than many of our own countrymen; hence, the rapid and phenomenal development of the leaders of medical thought in Berlin and Vienna.

The converse of this is equally true. The poorest diagnosticians of one's acquaintance are usually those who do not secure permission for the examination of their fatalities, and rarely if ever attend such an examination if secured by someone else. There are unfortunately those who are not above seeking to put their more aggressive and scientific colleagues in a false position in the eyes of the relatives.

Two or three decades ago for a physician to insist on the examination of the blood, urine, sputum or feces was to expose himself to the criticism of those physicians who claimed that their diagnostic acumen was such as to make such procedures unnecessary or even absurd. Those who today take this attitude are looked upon, even by the laity, as out of date and not worthy of confidence. Perhaps two decades hence, those who are unable to perform necropsies or to make use of the findings in a given case, will be viewed in exactly the same light. Their practice will gradually slip away from them, and when it is too late they will find that the progress of medical science has left them stranded, alone, and bemoaning the cruel fate that has deprived them of patronage in their declining years. Thus the biologic law of the survival of the fittest operates in our midst.

If a physician has done his best for a patient, and yet death occurs, the fault, if there be any, rests with the shortcomings of medical science and not with the individual concerned. Thus, with a free conscience, the physician has a right to inquire into the reasons for the failure. Death from disease is theoretically a therapeutic failure, and it is worthy of the medical idealist that he strive toward the relief of every patient entrusted to his care. The use of the necrospy is the best way to solve the problem, and to discover clews for future success.

In the same breath with which we give assent to this proposal we admit that we cannot be right in our judgment in every case. Omniscience is not a function of the human mind, as we tacitly admit when we provide lead pencils with erasers. Experience, however, lessens the frequency of mistakes. In the well-known principle of trial and error, we eliminate the errors by repeated trials. If the error is not discovered we continue with the trials indefinitely, as does the physician who continues to allow the undertaker to conceal his mistakes. Pursuance of this custom eventually banishes into the dim distance the sound principle of trial and error, replacing it with a phantom infallibility of judgment, which is the most dangerous form of self-delusion.

The symptoms of this psychosis would be interesting if the complaint were not so serious. It is insidious in onset, and is never realized by its victim. There soon ensues a feeling of self-satisfaction, of complacency, if not actual

euphoria. One patient after another consults this physician; each is examined in the shortest possible time, is given a diagnosis, receives a prescription and is urged to call again in a few days. Patients for the most part recover; hence the logical conclusion is reached that the diagnosis and treatment were correct. Each case tends to render the fixed idea still more firmly fixed. He has no consultations, hence there is never any difference of opinion. He is always correct. He has reached a stage of diagnostic infallibility which concedes no possibility of error. A superficial general examination of the portion of the body complained of, a touch on the pulse, a view of the tongue, and possibly a record of the temperature suffice for the completion of a positive diagnosis. Further examination is unnecessary and other consultations might as well be had over the telephone.

He is intensely resentful toward any physician who might consent to accept his patient in case the family demands consultation and he refuses. He considers this a personal insult, and his continued contemplation of this "unreasonable attitude" on the part of the family and "unprofessional conduct" of his associates bring him almost to the state of paranoia. His colleagues look on him as a difficult man to handle, and likely to develop intense dislikes on the least provocation. Those patients who are still loyal to him may readily admit that he is dictatorial, and they strive to avoid crossing him. This loyalty may, however, result in definite injury to the patient, or even an unnecessary fatality.

Failure to recover even when recovery was predicted, or the appearance of unlooked-for symptoms is brushed aside as of little or no importance. The possibility of there being other diseases present, or that the diagnosis is entirely wrong, is not considered. He has a routine remedy for every cough, a stock prescription for every form of headache, a specially compounded pill for pain and his own private liniment that is good for every muscle and joint disturbance. Abdominal pain is always constipation and calls for drastic catharsis. Even strangulated hernia, intussusception, and appendicitis are treated in this routine manner. An occasional fatality is an intervention of the Lord, and the physician is not in the least responsible. A "touch of pneumonia" or a "complication" may close the picture, never the rupture of a gangrenous appendix or peritonitis.

This is an extreme case, but one not so rare even in our day. There are milder cases of the same complaint, but all those so afflicted have in common one essential feature. They have no place in their life or profession for the principle of trial and error, or for the necropsy which is the only means of autocorrection. There is no known method of cure which the afflicted physician would agree to take. Prevention is the sole means of treatment of which we know. This consists of habitual direct observation of pathologic processes, and the suppression of an ultravivid clinical imagination. The remark of Francis Bacon that medical science is more professed than labored, applies only too commonly today.

You would be extremely resentful if I possessed the gift of prophesy and could tell you that certain ones here tonight would conform to this picture

twenty years hence. No morphine habitué would become one if he could see the end from the beginning. But I warn you that this disease will come upon even you unless you protect yourself and your professional career by the only known means of prophylaxis. Remember that its onset is insidious, and that you will probably not recognize its presence when or if it does come upon you.

The natural question to ask is, how can one perform necropsies if the services of a professional pathologist in the community where one expects to practice cannot be secured? I would answer, you expect to make of yourself a surgeon or an internist or perhaps some other specialist. You expect to work to that end until you have accomplished the desired result. Do you think Addison or Bright or any of those men whose names have been mentioned hesitated for this reason? Each was his own pathologist; you can be your own. When there is a will there is a way. This way will be explained a little later. This is merely an excuse, not a reason, and having had the way pointed out, persistence in this excuse should warn you that you might readily succumb to this dread disease.

As a conscientious physician you have no mortal right to ask permission for that which you would not permit for yourself or a member of your own family. You may not be asked to commit yourself in public, but this is a challenge to you, that you cannot evade. You must decide what you will do about the matter so far as your future career is concerned. Putting the matter in more concrete form, there are two questions which you are called on to face: First, will I let it be known to my family that in the event of my death I desire that a postmortem examination shall be held, and put this so strongly that my relatives will feel it their duty to carry out my wishes if this is possible? They should seek someone to perform this examination, even if the attending physician did not of his own volition make this request. Second, will I obligate myself to urge an examination on a member of my immediate family, and to use my influence to secure such permission in the case of a more distant relative?

In 1914 Dr. C. D. Spivak, a Jewish physician of Denver, wrote an article entitled "Postmortem Examinations Among the Jews: An Historical Sketch and a Plea to Jewish Physicians." This states that for the previous ten years the Medical Advisory Board of the Jewish Consumptive Relief Society had consistently carried out the policy of seeking permission for necropsy in every fatal case in which the clinical symptoms were at all vague. This was in the face of opposition of which we have little conception. A society was formed to oppose this action, and to fight against this modern tendency by every known method, seeking to invoke both church and civil law. This article is a strong plea to rabbis and Jewish physicians to modify the current beliefs and to nullify obsolete laws. Unquestionably Spivak's influence and example have borne much fruit, at least among the more liberally minded Jews.

Dr. Spivak died recently, and the example that he set is worthy of permanent record which all may read who have even the slightest interest in necropsies and medical advancement. In his will he directed that his body be

dissected by an equal number of Jews and Gentiles, and that thereafter his skeleton be articulated and sent to the University of Jerusalem. His wishes have been carried out. A special necropsy was arranged, preceded by ceremonies of appropriate nature, at which there were several addresses. His example has made it possible to link more closely the Medical School and the National Jewish Hospital for Consumptives. The pathologic staff of one conducts the examinations for the other. Thus does the courageous example of one man bear fruit that will benefit untold numbers.

Those who are willing to go with me so far as to agree to necropsy for themselves and for their relatives might be inclined to go one or two steps farther. If one believes in necropsies himself, he should do all he can to persuade others to do likewise. Just how this may be accomplished varies with the person and the situation in which he finds himself. There are several possibilities:

1. *General Practitioner in a Small City.*—I can do no better than to call to your attention the experience of Waite in Texas. Here was a man who so thoroughly believed in necropsies that he sought until he found a satisfactory solution for his problem. He wrote two articles entitled, "Home Postgraduate Work by Means of Postmortem Examinations" and "An Experiment in Postgraduate Work at Home." In short, this experiment was the banding together of a small group of physicians for the purpose of securing as many necropsies as possible, and their detailed study. One physician recorded 100 per cent permissions in his practice, and others reported high percentages. This group reported on the results of ninety-five examinations. The cost, which was slight, was prorated, but eventually it was found advisable and worth while to employ a pathologist to assist in the work. They found it worth their energy, time and money; so would you.

2. *General Practitioner but Connected with a Hospital Staff.*—You may let it be known that you are in favor of necropsies by setting a good example with the patients on your own service. Your own activity is likely to encourage others less courageous. If your hospital is to secure recognition as a suitable place for internships it is now necessary to have a pathologist connected with the staff and have at least a certain specified but ever-increasing percentage of necropsies on fatalities. This is a strong lever with which to lift the staff out of a state of lethargy and indifference.

3. *Specialist with Services in One or More Hospitals.*—Your support can be given more strongly to those hospitals that meet the requirements of the American Medical Association than to those that do not. You can seek to reeducate those staff men who oppose necropsy on their patients, and strive to counteract their influence in this respect in the conduct of the hospital. By this action the hospital and all concerned will be benefited. Men who consistently oppose are likely to be less progressive and less scientific, and their places can be taken with profit by those who are capable of making much better use of the hospital facilities.

Assuming that you have done everything possible to secure permission for necropsy in every case, the problem is not entirely solved. Incidental men-

tion has already been made of the desirability of having a necropsy well performed. There are three factors involved in this phase of the question:

1. It is axiomatic that the more skillful the pathologist the more valuable will be the necropsy to all concerned. Some facts can be ascertained by anyone, while others require good technic, nice discrimination and skillful interpretation. The best pathologist cannot be secured for every case, yet it is necessary to seek to improve the service in every possible way.

2. The necropsy must be performed in such a way as to assist rather than hinder the embalmer in his work. This is a technical matter with which every pathologist should be familiar or, if he is in doubt, the embalmer should be consulted. Vessels cut too short, or left unligated, often cause much avoidable difficulty, and lay the foundation for later opposition on the part of the undertaker.

3. An interview should be held with the relatives, during which they should be fully informed of the nature of the disease, its course, the nature and reasons for any operation performed, and an interpretation given of the conditions leading to the death. This should be frank and straightforward, full and explicit, yet simple and devoid of medical terms, and carried out with the full consciousness of a duty faithfully performed. It is often an advantage to have someone hold this interview who has not been directly connected with the case, such as the pathologist or the superintendent, and yet one who has sufficient knowledge of details to discuss the case with ease and precision.

Special attention paid to the relatives at the time of their acute grief and a detailed explanation of the whole situation may readily dispel disturbing thoughts and correct wrong impressions with the result that the full confidence of all concerned is retained. A physician who has done his duty is entitled to retain the full confidence and respect of the relatives of a patient whom he has lost, and this he can do by facing the issue fairly and squarely in the manner suggested.

I trust that the foregoing presentation has not been without effect on you. Theoretical assent to these principles should have given place to active advocacy. Nominal agreement with these facts should have been replaced by a conviction that necropsies are essential to the progress of our science.

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EFFECT OF STORAGE ON CHEMICAL AND ANTISEPTIC PROPERTIES OF SILVER PROTEIN SOLUTIONS*

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ACCORDING to the United States Pharmacopoeia, Tenth Revision, solutions of silver protein, both mild and strong types, should be freshly prepared and preserved in well closed containers, protected from the light. A search of the literature for facts on which these specifications might be based showed such a mass of conflicting evidence that an investigation was undertaken to determine, if possible, the changes that occur in silver protein preparations during storage.

The strength of silver protein solutions was determined by three methods, the germicidal power, the inhibitory action against yeast, and potentiometric titrations of the silver ion.

GERMICIDAL POWER

It is generally accepted that the germicidal power of argyrol and protargol is not great, but no two workers agree on the germicidal efficiency of these silver proteinates.

Derby[1] found that, while protargol in 2 per cent to 4 per cent solutions was effective against pneumococcus in one minute and against Staphylococcus aureus in ten minutes, a 50 per cent argyrol solution did not kill Staphylococcus aureus in two hours. Marshall and Neave[2] found that 0.025 per cent protargol killed Staphylococcus aureus in ten minutes. Verhoeff[3] reported that 12 per cent argyrol would not kill Staphylococcus aureus in one hour nor would 50 per cent argyrol in one-half hour. Kelly[4] found that 0.5 per cent protargol killed Staphylococcus aureus in ten minutes, while 50 per cent argyrol was ineffective in twelve hours. Post and Nicholl[5] found that 10 per cent argyrol killed B. typhosus in one minute but was of little value against the streptococcus, pneumococcus, or gonococcus. Martindale and Westcott[6] gave figures very similar to those obtained by Derby. Culver,[7] using the gonococcus as a test organism, found that ¼ per cent argyrol or silvol and ⅛ per cent protargol would kill in ten minutes. Dakin and Dunham[8] state that Staphylococcus aureus gave growth after a six-hour exposure to 5 per cent argyrol, but that there was a diminution in the number of colonies in five minutes. Young[9] found that 10 per cent argyrol killed Staphylococcus aureus in urine in five minutes, while 1 per cent protargol failed to kill in one hour. Lancaster[10] showed that 10 per cent argyrol was capable of killing Staphylococcus aureus in one minute when only a small amount of the test culture was used in serum. Cheney[11] tested argyrol and protargol and obtained results from which he concluded that neither preparation was of value in the presence of pus, that

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the presence of clumps of bacteria in the test culture greatly affected the results and was responsible for the different findings, and that 1 per cent argyrol is as effective as a 25 per cent solution.

This conflict in results may be ascribed to the lack of any standardized method of testing. The test organisms were grown on various media with a consequent difference in resistance. The amounts of culture used in the tests ranged from 0.5 c.c. to that adhering to a straight needle. The argyrol and protargol have been diluted in various ways, with distilled water, with salt solution, with serum, with hydrocele fluid, and with blood. The treated organisms have been subcultured on numerous media, including agar slants, blood agar, and broth. The age of the solutions was considered by some and not by others.

STABILITY OF SOLUTIONS

The results of investigations on the keeping qualities of silver protein preparations reported in the literature show a somewhat better agreement.

Brüning,[12] from a study of the changes in alkalinity and appearance on storage, decided that protargol solutions should be freshly prepared for use, but that they might be preserved for a short period in a cool dark place. Culver[7] tested several silver protein preparations exposed to daylight for two months and found that the decrease in strength against gonococcus was from 50 to 75 per cent. He states that the loss in strength begins as early as the third day and is almost directly proportional to the age of the solution. Sollmann and Pilcher[16] tested the variations in strength of a number of silver protein solutions over a period of one year by means of the inhibitory action on the growth of yeast. They found that the strong type (protargol) decreased in strength, while the mild type (argyrol and silvol) increased. Schlee and Thiessenhusen[17] stored a number of preparations for periods of nine to seventeen months and determined the concentration of silver ion by means of the potentiometer. They found that during this period all solutions developed precipitates, causing a loss in the total quantity of silver in solution. The silver ion concentration decreased in some cases and increased in others. All solutions showed an increase in electrical conductivity. The total number of colloid particles suffered diminution in every case. The size of the particles increased in some cases and remained unchanged in others.

YEAST METHOD

The yeast method, several modifications of which are described in the literature,[14, 18, 19] is based on the theory that the inhibitory action of any silver protein on the growth of yeast is dependent upon the silver ion concentration and is proportional to its germicidal power. This theory has recently been questioned by Taylor,[20] who gives data showing that the most strongly germicidal compound he worked with had practically no inhibitory action on the growth of yeast.

Our experiments with this method have convinced us that the inhibitory action of the silver proteins on the growth of yeast cannot be compared to that produced by silver nitrate. For example, when we approach the inhibitory

concentration of silver nitrate, a slight increase in silver ion will cause a small decrease in the amount of gas produced by the yeast in a given period. On the other hand, when the concentration of silver protein is increased to give a corresponding increase in "silver ion," there is a marked decrease in the volume of gas. The data obtained over the period of study were so erratic as to be valueless for the purposes of this investigation. The results of this work will be published elsewhere.

POTENTIOMETRIC TITRATION OF SILVER ION

The potentiometric determination of silver salts is based on the theory that the potential developed by a silver electrode immersed in a solution of a silver salt is proportional to the concentration of silver ion. The silver ion concentration may be determined either directly from the voltage produced against standard electrodes or by titrating the solution with some ion that will reduce the concentration of silver ion. The end-point of the titration is determined by means of the enormous change of voltage shown when the last of the silver passes into the less ionized or more insoluble compound. This method does not, in fact, show the amount of the silver ion but it does show the quantity of silver more ionizable (or soluble) than the product of the reaction.

Neergaard[21] obtained the same values by titrating various organic silver preparations potentiometrically with chlorides, bromides, iodides, and sulphides. He found that when 3 c.c. of normal acetic acid were added to 100 c.c. of a protargol solution he was able to titrate 90 per cent of the silver instead of the 66 per cent previously obtained. He was unable to account for this, or for the ability to titrate neosilversalvarsan with sodium sulphide as the silver ion concentration of neosilversalvarsan solutions is of the same order as that of a potassium silver cyanide solution. He mentions one silver compound, hegonon, which was dissolved in potassium cyanide and the silver correctly titrated with sodium sulphide.

Treadwell, Janett and Blumenthal[22] titrated silver preparations with chlorides and sulphides. They report some cases in which the chloride titrated only a portion of the silver and the sulphide titrated all. In other cases the chloride titrated all the silver.

Blumenthal[23] concluded that titration with sodium chloride would show ionic silver, that titration with sodium sulphide would show both ionic and complex silver, and that the total silver should be determined after oxidation with sodium peroxide.

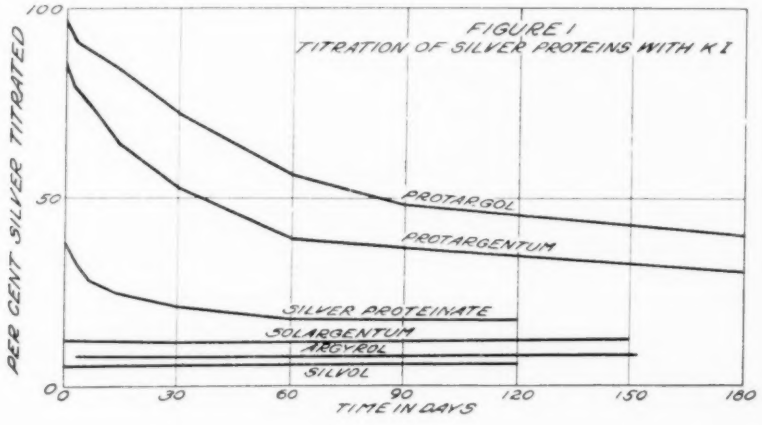
Smith and Giesy[24] titrated several silver preparations with standard solutions of hydrochloric acid, hydriodic acid, sodium chloride, and potassium iodide. They observed differences in the shapes of the titration curves, but all reagents gave the same end-point. In the case of the mild type, solargentum, this amounted to 11 per cent of the total silver and in the case of the strong type, protargentum, to 81.5 per cent of the silver. The curve with the mild type, using hydrochloric acid, was so irregular as to be meaningless. The authors were unable to ascribe a reason for this.

Kolthoff and Tomicek[26] titrated protargol containing 7.87 per cent silver

with standard solutions of sodium chloride, potassium bromide, and potassium iodide. They were unable to obtain an end-point with sodium chloride. Potassium iodide gave a slightly higher figure than potassium bromide. Upon the addition of sulphuric acid before titration (4 c.c. normal acid in 50 c.c. solution), sodium chloride and sodium bromide gave approximately 7 per cent silver. Potassium iodide, on the other hand, showed one break in the titration curve equivalent to 7.08 per cent and another equivalent to 7.91 per cent silver. It is stated that the difference is due to the presence of silver chloride in the original sample. They conclude that all of the silver in protargol can be titrated with potassium iodide in the presence of sulphuric acid.

METHODS EMPLOYED

A 0.02 N solution of potassium iodide was chosen as the reagent for the titrations. When this was added to the silver protein solution a gradual change in voltage was produced, equilibrium being obtained quickly in most

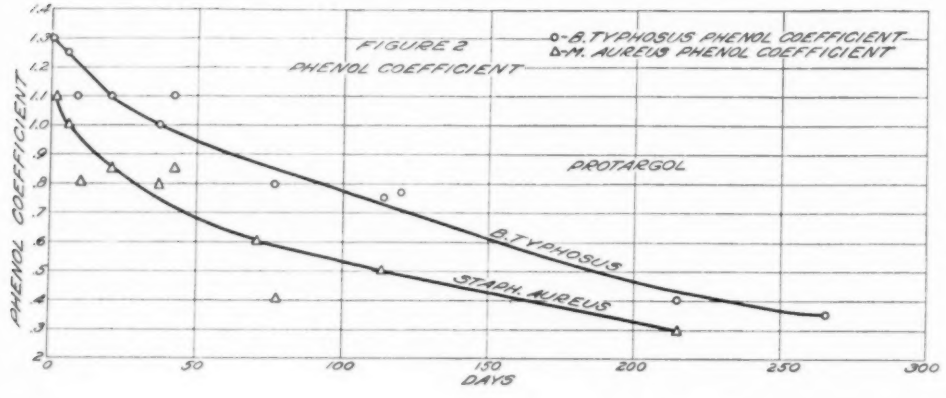


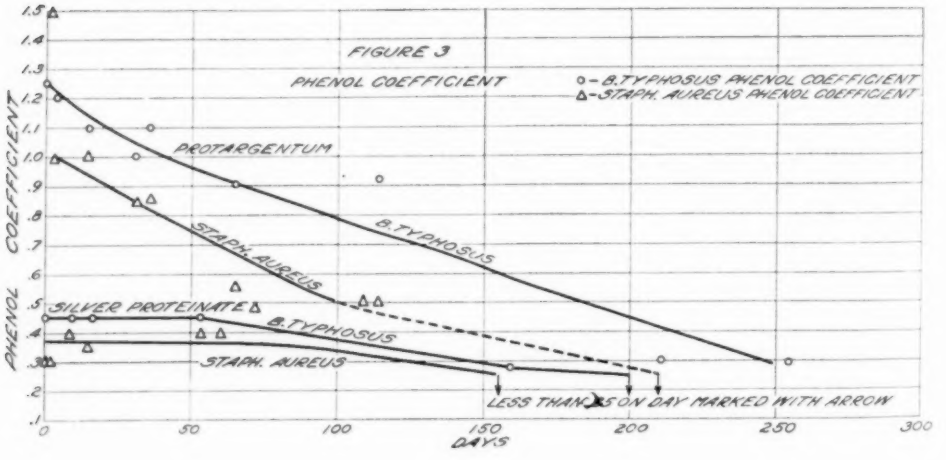
cases, and a very sharp break was shown in the titration curve at the end-point. The solution remained clear throughout. Experiments using the method of Kolthoff (4 c.c. normal sulphuric acid in 50 c.c. solution) were unsuccessful. It was found that each addition of potassium iodide produced an enormous change in voltage. On standing, the voltage gradually returned to nearly the original value, fifteen to twenty minutes being required for it to reach equilibrium. Near the end-point the product was completely precipitated as a brown mud, leaving a clear colorless supernatant liquid. What appeared to be the end-point gave a slightly higher value for silver ion than was obtained in neutral solutions.

Several titrations of protargol were made after the addition of acetic acid as described by Neergaard. These titrations showed about 0.1 per cent more silver than was obtained from neutral solutions.

The method followed in the bacteriologic tests is that described by G. F. Reddish,[29] and used in the Food, Drug and Insecticide Administration for testing disinfectants and antiseptics. The organisms used were Bacillus typhosus (Hopkins strain) and Staphylococcus aureus. Both are laboratory

strains whose resistance to phenol has remained constant for about five years, and comes well within the limits prescribed by the test. This method of determining the phenol coefficient was used because of its simple technic and consistent results as compared with Hygienic Laboratory and the Rideal Walker Methods. By expressing results of the bacteriologic tests in terms of the phenol coefficient the germicidal power of these solutions is compared with a standard germicide, and the phenol figure also serves as a control.





EXPERIMENTAL WORK

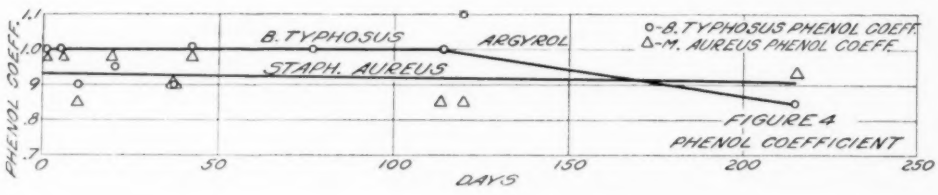
Approximately 5 per cent solutions in distilled water of six brands of silver protein, three being of the mild type and three of the strong type, were prepared. The silver contents of these solutions were determined by evaporation to dryness, igniting to destroy the organic material, dissolving in nitric acid, and titrating with standard thiocyanate. The solutions were stored in amber bottles, with cork stoppers, and at fixed times samples were tested against yeast and bacteria, and titrated with 0.02 N potassium iodide solution.

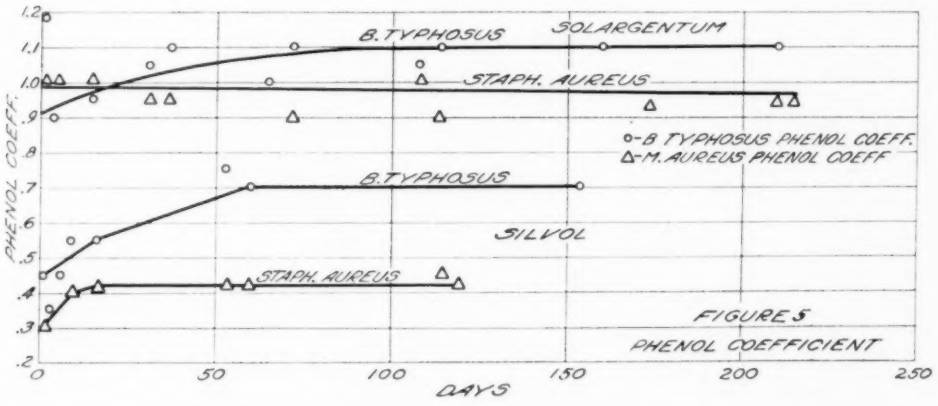
The results of the experimental work are shown graphically in Figs. 1, 2, 3, 4, and 5. The titration values for the strong silver proteins (protargol,

protargentum, and silver proteinate Mallinckrodt) decreased rapidly at a fairly uniform rate, and those for the mild type (argyrol, silvol, and solargentum) increased very slightly. The results when the yeast inhibition method was used, although rather erratic, indicated the change with age shown by the titration method. The mild type increased and the strong type decreased in strength with age.

Plotting the phenol coefficients of these solutions over a period of from five to eight months, curves are obtained which are quite similar to those representing the titration values.

As shown by the points which do not fall on the line, slight deviations were obtained from the smoothed curve. In as much as the limit of error in





determining the phenol coefficient is one or two-tenths of a point, the aberrant figures may be worthy of no consideration. On the other hand, while the grafts magnify the amplitude of these deviations, the paths followed by the Staph. aureus and the B. typhosus coefficients, in at least two of the solutions, are of such close similarity as to point to a true rise and fall. Further work is in progress on this point.

The similarity between the bacteriologic curves, using either the Staph. aureus or the B. typhosus phenol coefficient, is not in agreement with the results of Taylor,[20] who found that the germicidal values against the two organisms did not correspond. The corresponding decreases with age of the germicidal and tritratable potency of these solutions would seem to confirm the theory of Sollman and Pilcher that the activity is due to the silver ions, except that the relative magnitude of the germicidal powers of the strong and mild types do not correspond at all with the titration values. In fact, our results indicate that the germicidal efficiency of preparations of the mild

type of silver protein is equal to, and in old solutions higher than, that of the strong type.

During the period of observation, sediment in varying quantities appeared in three of the six solutions. Accordingly, the total silver content of the solutions was redetermined at the end of the investigation. In no case was a decrease of over 2 per cent of the total silver observed. Much difficulty was experienced in all but two cases in an effort to eliminate a growth of mold, identified as Penicillium elongatum Bainies,* on the corks of the bottles. The mold was repeatedly cleaned from the cork, which was then washed with the silver protein solution. This was not successful, however. No explanation can be given for its absence in the two samples, one of each type, as all were kept under identical conditions.

The inhibitory action of these preparations is in marked contrast to their germicidal efficiency. The inhibitory action in this case was considered to be the absence of growth in a mixture of antiseptic and nutrient broth after five days incubation at 37° C. While the germicidal action of the freshly prepared solutions is comparable with that of phenol, their inhibitory power on the growth of B. typhosus was approximately ten times as great, a 1-7000 protargol solution and a 1-10,000 solution of argyrol being just sufficient to prevent growth of the B. typhosus. Due to the extremely slow and scanty growth of Staphylococcus aureus in an unfavorable medium no exact figures were obtained for this organism. The inhibiting concentrations were in approximately the same ratio but very much lower than in the case of B. typhosus. In this case the change with age, if any, was too slight to be measured at such high dilutions.

DISCUSSION OF RESULTS

The inhibitory action of silver proteins against yeast apparently cannot be used as a criterion of their strength. It is extremely doubtful whether the potentiometric titration method, or the electrical measurements of silver-ion concentration, actually furnish a measure of the silver ion. The ability to completely precipitate the preparation with iodide ion in presence of an excess of sulphuric acid tends to confirm this belief. Again, two samples of the same material purchased at different times from the same manufacturer assayed the same amount of silver, but titration with iodide showed different percentages of silver ion, assuming that silver ion is being titrated. This variation in individual products has recently been mentioned by another investigator,[30] who says, "It is evident that the difference between samples of the same brand is as great as, or greater than, the difference between individual brands."

Two theories have been advanced to explain the action of these preparations. One is that the strength depends on the concentration of the silver ion. Another[25] is that the size of the particles may have an effect on their potency. This has been shown to be true for certain other compounds.[27] Although there is evidence to support both theories, we believe that neither can satisfactorily

*Identified by Dr. M. B. Church of the Microbiology Laboratory, Bureau of Chemistry & Soils.

explain the results obtained in these investigations. Gros[28] demonstrated that the presence of hydroxyl ions greatly increased the germicidal properties of silver preparations. Schlee and Thiessenhusen found that the size of the particles did not always change with age. It is therefore certain that the loss in silver "ion" cannot be counter-balanced in every case by a change in the size of the particle, although this offers a possible explanation for the fluctuations in potency. Finally, when two such closely related substances as mercuric chloride and mercuric iodide show wide variation in their germicidal values,* it seems hardly plausible to assume that the action of silver nitrate and that of silver proteins with widely differing protein radicals, may be compared on the basis of the silver ion.

CONCLUSIONS

According to all methods used for testing, solutions of strong silver protein deteriorate appreciably in six months while solutions of the mild type do not. Neither the yeast inhibition test nor the potentiometric titration of silver ion yields an index to the relative germicidal value. There is reason to believe that the silver ion is only partially responsible for the germicidal efficiency.

Both types of silver protein were found to have approximately the same strength in freshly prepared solutions and the concentrations ordinarily used in medical practice are germicidal.

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*Solutions of potassium mercuric iodide contain less mercury ion than mercuric chloride solutions of the same concentration. While the iodide is less germicidal than the bichloride towards Staphylococcus aureus it has five times the strength against B. typhosus.

THE FAILURE OF PITUITARY SUBSTANCES TO INFLUENCE THE BASAL METABOLISM OR THE SPECIFIC DYNAMIC RESPONSE TO FOOD IN A NORMAL SUBJECT*

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IN THE literature exist numerous contradictory reports of the results of feeding and injection experiments with preparations of the pituitary gland. It is generally conceded that pituitary gland preparations do not exert their optimal physiologic action when administered by mouth although Rees and Olmstead[1] have reported some success in the treatment of a case of diabetes insipidus when posterior lobe preparation was administered orally in salol-coated capsules. Likewise, Morris and Weiss[2] treated successfully a case of dyspituitarism with the administration of such extracts by mouth. Pituitrin administered orally was found by Donaldson[3] to be of value in cases of uterine hemorrhage. Grabfield and Prentiss[4] observed no influence on the nitrogen metabolism when pituitary preparations were administered orally and intramuscularly. Knaus,[5] however, found that pituitrin by mouth affected the uterus of a cat within eight minutes after its administration.

Other investigators have been primarily concerned with the effect of pituitary preparations on the respiratory exchange. Bernstein and Falta[6] observed in this connection a difference in the actions of posterior and anterior lobe preparations. In either normal individuals or patients with hypophyseal or thyroid disease, injections of posterior lobe extracts produced a moderate increase in the values for carbon dioxide output and oxygen consumption without affecting the respiratory quotient while injections of anterior lobe substance produced a lowering of the basal metabolism accompanied by an increase in respiratory quotient.

Kestner, Liebeschutz-Plaut, and Schadow[7] observed an increase in the specific dynamic response to foods, after oral administration of anterior lobe preparations (präphyson), while no change was evident when a mixture of posterior lobe preparations (hypophysin, physormon, and pituglandol) was fed either to normals or to cases of Fröhlich's syndrome (dystrophia adiposogenitalis). These investigators could not confirm, however, the earlier observations of Liebesney[8] that the feeding of anterior lobe preparations may lead to a decrease in the specific dynamic response to food.

Several investigators of the German school (Plaut, et al.) have attempted to use the increase in the metabolism of an individual after a standard meal as a diagnostic test. Upon the magnitude of this response, differential diag-

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noses have been based with especial attention directed toward the hypophysis. It therefore became of interest to determine,

1. The influence of the administration of various pituitary preparations upon basal metabolism, and
2. The effect of the administration of these preparations upon the increase in metabolism following the ingestion of a standard meal.

THE BASAL METABOLISM

Our interest in the influence of preparations of pituitary gland upon the basal metabolism was first stimulated by the response of a patient with a pituitary tumor to the administration of whole pituitary gland substance (Wilson). This patient (D. R.), a male, aged fifty-two years, had the following symptoms: weakness, somnolence, headaches, right lateral strabismus, scanty beard and absence of axillary hair, feminine distribution of pubic hair, unusual smoothness and softness of the skin, right optic atrophy, and facies suggestive but not typical of acromegaly. Roentgenograms showed marked enlargement of the sella turcica. His basal metabolism was, –18.6 per cent (DuBois). This patient was under observation in the hospital for three months and during a part of that time received whole pituitary gland substance (Wilson) in doses approximately one gram per day (Gr. IV three times a day). Table I represents his basal metabolism during the period of observation.

TABLE I

DATE	BASAL METABOLISM (DU BOIS) PER CENT	PULSE RATE	REMARKS
12/23/25	–18.6	66	
12/26/25	–18.3	68	
1/ 4/26	–16.0	68	
1/23/26	+ 2.0	66	Pituitary whole gland substance 1.0 gram daily since 1/8
2/ 9/26	– 8.0	78	
2/17/26	–17.0	72	Medication discontinued since 2/10
2/23/26	–23.0	68	Pituitary whole gland substance 1.0 gram daily starting 2/24
3/ 4/26	–19.0	70	
3/11/26	+ 3.0	110	Patient feeling poorly

These results suggest a possible effect of pituitary therapy upon the basal metabolism of this individual. The metabolism increased from – 16 per cent to + 2 per cent within a period of two weeks during which the patient received pituitary extract one gram daily by mouth. Seventeen days later the metabolism was – 8 per cent and the pituitary extract was discontinued. Within the next thirteen days the basal metabolism had fallen to – 23 per cent, a value below the initial rate determined. Pituitary extract was again administered in the same amount. Eight days later the basal metabolism was – 19 per cent; a week later + 3 per cent. At about this time, however, the patient began to feel poorly and developed a bronchopneumonia, dying eight days later. The pituitary tumor was confirmed at autopsy.

The fact that the second administration of pituitary substance failed to effect the basal metabolism of this individual after eight days raises a ques-

tion in regard to the cause of the increase observed following the initial administration and leaves a doubtful impression regarding the effect of the substance upon the basal metabolism.

It was therefore of interest to study the effect of the administration of pituitary gland substance to a normal individual, on a constant diet who could be studied carefully over a long period of time. The subject used was a physician, twenty-seven years old, 182 cm. tall and weighing 72 kilo. He

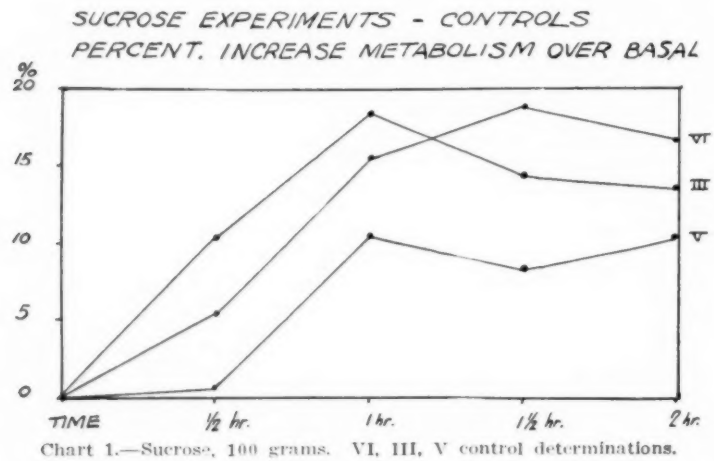

Chart 1.—Sucrose, 100 grams. VI, III, V control determinations.

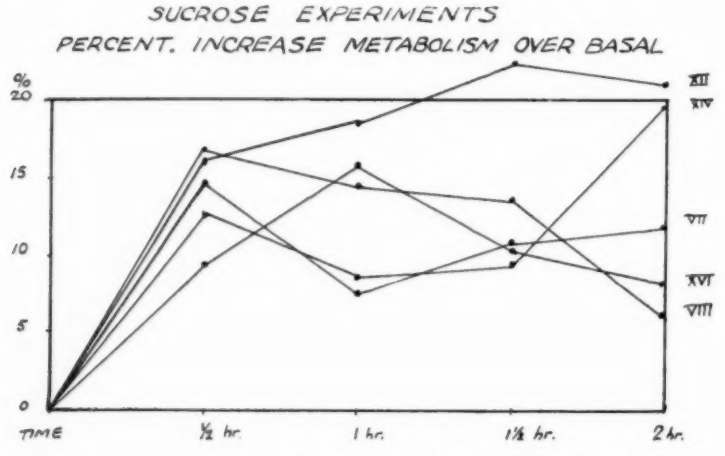

Chart 2.—Sucrose, 100 grams. XII, Antuitrin, intramuscularly for five days. XIV, Posterior lobe extract, intramuscularly for six days. VII, Whole pituitary gland, orally for seven days. XVI, Posterior lobe extract, intramuscularly for eleven days. VIII, Whole pituitary gland, orally for fifteen days.

was free from symptoms of any sort and worked daily as a member of the hospital staff. A physical examination at the beginning of the experiment was entirely negative. In spite of the failure of clinical symptoms of any sort, this individual was consistently found to have a low basal metabolism. Determinations were made with a Tissot spirometer at intervals during a control period of thirty days with the following results: −18.0 per cent,

– 19.0 per cent, – 21.8 per cent, – 18.5 per cent, – 14.2 per cent, – 19.0 per cent, and – 20.0 per cent. These results agreed also with determinations made with the Benedict-Collins-Roth apparatus. In explanation of these consistently low results we can only offer the suggestion that our subject had been leading a rather sedentary life for the past eight years, and also point out the observation made by Sturgis[9] in this connection, "It has been noted that the basal metabolism in a series of normal individuals, all house officers or medical students in many instances tended toward the extreme lower border of normal; that is, between 10 and 16 per cent below the average. It may be that these individuals in an endeavor to cooperate attain almost complete relaxation which is similar to that observed during sleep, and this might well account for the slightly diminished but not abnormal metabolism." Almeida[10] has done some interesting experiments demonstrating that inactivity is able to produce a lowering of the basal metabolism.

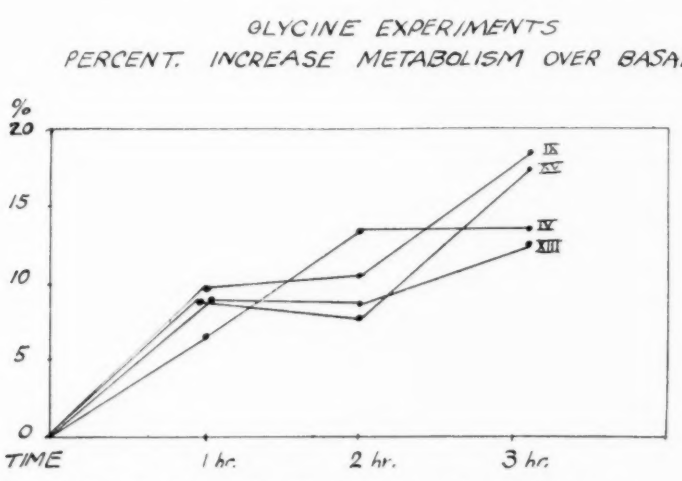

Chart 3.—Glycine, 50 grams. IX, Whole pituitary gland, orally for sixteen days. XV, Posterior lobe extract, intramuscularly for seven days. IV, Control. XIII, Antuitrin, intramuscularly for seven days.

Since our subject presented no other abnormalities and our interest lay in the effect of pituitary substance on the basal metabolism, it was decided to continue the experiment.

EXPERIMENTAL DETAILS

The Tissot method of determining the basal metabolism was employed and the gas analysis carried out by means of a carefully calibrated Haldane or Henderson apparatus. The determinations of basal metabolism were made under the usual standard conditions. The subject was placed on the following diet which contained a gram of protein per kilo: protein 72, fat 190, and carbohydrate 280 grams, calories 3118.

Whole pituitary substance (Wilson—in special coated capsules said to be resistant to the action of the gastric juice but soluble in the duodenal juice) was administered by mouth as follows: 1 gram daily for seven days

followed by 3 grams daily for sixteen days. For an anterior lobe substance antuitrin (Parke-Davis) was injected intramuscularly in 4 c.c. amounts for seven days, while pituitary substance obstetrical (Wilson) in the same quantity was administered intramuscularly for eleven days.

RESULTS

Table II indicates the results obtained during the above periods. Neither the respiratory quotient nor the basal metabolism were markedly influenced by the oral administration of whole pituitary substance over a period of sixteen days, of antuitrin administered intramuscularly for seven days or by posterior lobe substance injected intramuscularly for eleven days.

THE SPECIFIC DYNAMIC RESPONSE TO SUCROSE AND GLYCINE

In addition to the determinations of the basal metabolism recorded above, a study was made of the increase in heat production following the ingestion of a standard meal and the effect of these various pituitary preparations on the magnitude of that increase. The protein meals used by Plaut,[11] Wang, Strause, and Saunders,[12] Liebesney,[13] and others may be criticized on the ground that they contained considerable amounts of fat and carbohydrate as well as protein, and that therefore the rate of absorption may have played an important part in the time at which the greatest response was observed.

In this connection it might be well to mention one experiment we performed on a patient, using a test meal similar to that described by Plaut with

TABLE II

EFFECT OF ADMINISTRATION OF PITUITARY SUBSTANCES ON BASAL METABOLISM

EXP. NO.	DATE	O_2 PER MIN. C.C.	CO_2 PER MIN. C.C.	R.Q.	CAL. PER SQ. M. PER HR.	BASAL METAB. PER CENT	PULSE	REMARKS
I	11/ 9/26	215	167	0.78	32.2	−18.5	81	Control period
II	11/13/26	206	161	0.78	30.9	−21.8	70	Control period
III	11/18/26	215	168	0.78	32.2	−18.5	81	Control period
IV	11/24/26	214	164	0.77	32.0	−19.0	75	Control period
V	11/27/26	227.5	170	0.75	33.9	−14.2	72	Control period
VI	11/30/26	208	167	0.80	31.4	−20.5	74	Control period
VII	12/ 7/26	214	170	0.80	32.2	−18.5	75	Whole pituitary gland 1 gm. daily since 12/2
VIII	12/22/26	223	174	0.78	33.4	−15.4	72	Whole pituitary gland 3 gm. daily since 12/7
IX	12/23/26	214	168	0.78	32.1	−18.7	73	Whole pituitary gland 3 gm. daily since 12/7
X	1/11/27	205	169	0.82	30.6	−22.5	71	Control. No pituitary since 12/23/26
XI	1/18/27	211	166	0.79	31.1	−21.3	73	Control. No pituitary since 12/23/26
XII	1/23/27	202	159	0.78	29.9	−24.3	73	Antuitrin 4 c.c. daily since 1/18
XIII	1/25/27	208	164	0.79	30.9	−21.8	70	Antuitrin 4 c.c. daily since 1/18
XIV	2/ 3/27	228	168	0.74	33.3	−15.7	73	Posterior lobe extract 4 c.c. daily since 1/25
XV	2/ 4/27	206	163	0.79	30.5	−22.8	70	Posterior lobe extract 4 c.c. daily since 1/25
XVI	2/ 8/27	218	174	0.80	32.4	−18.0	80	Posterior lobe extract 4 c.c. daily since 1/25

TABLE III

THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE

EXP. NO.	BASAL METAB. PER CENT	RESP. QUOT.	METABOLISM AFTER INGESTION OF SUCROSE								REMARKS
			½ HOUR		1 HOUR		1½ HOUR		2 HOURS		
			PER CENT INCREASE OVER B. M.	RESP. QUOT.	PER CENT INCREASE OVER B. M.	RESP. QUOT.	PER CENT INCREASE OVER B. M.	RESP. QUOT.	PER CENT INCREASE OVER B. M.	RESP. QUOT.	
III	−18.5	0.78	10.4	0.92	18.2	0.94	15.2	0.93	13.7	0.93	Control
V	−14.2	0.75	0.2	0.91	10.7	0.90	8.9	0.86	11.2	0.93	Control
VI	−20.5	0.80	5.1	0.92	15.4	0.92	19.4	0.91	16.1	0.93	Control
VII	−18.5	0.80	15.0	0.96	7.6	0.90	10.6	0.91	11.9	0.91	Whole pituitary gland 1 gm. for 7 days
VIII	−15.4	0.78	16.7	0.95	14.6	0.96	13.4	0.82	7.1	0.89	Whole pituitary gland 1 gm. for 15 days
XII	−24.3	0.78	16.2	0.85	18.5	0.89	22.3	0.93	21.0	0.92	Antuitrin 4 c.c. intramuscularly for 5 days
XIV	−15.7	0.74	12.7	0.89	8.1	0.88	9.9	0.95	19.2	0.90	Posterior lobe 4 c.c. intramuscularly 6 days
XVI	−18.0	0.80	9.1	0.95	15.5	0.95	10.1	0.90	8.1	0.93	Posterior lobe 4 c.c. intramuscularly 11 days

TABLE IV

THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE

EXP. NO.	BASAL METAB. PER CENT	RESP. QUOT.	METABOLISM AFTER INGESTION OF GLYCINE						REMARKS
			1 HOUR		2 HOURS		3 HOURS		
			PER CENT INCREASE OVER B. M.	RESP. QUOT.	PER CENT INCREASE OVER B. M.	RESP. QUOT.	PER CENT INCREASE OVER B. M.	RESP. QUOT.	
IV	−19.0	0.77	6.8	0.81	13.9	0.88	13.4	0.81	Control
IX	−18.7	0.78	9.8	0.82	10.3	0.79	18.7	0.83	Whole pituitary glands 3 gm. daily 16 days
XIII	−21.8	0.79	9.8	0.84	8.9	0.88	11.7	0.87	Antuitrin 7 days
XV	−22.8	0.79	9.4	0.86	7.9	0.88	17.1	0.84	Posterior lobe 7 days

the exception that a small amount of barium sulphate was incorporated with the chopped meat. It is of interest that at the end of three hours there was still visible by means of the fluoroscope a bolus of barium-containing food, this in a patient without recognizable gastrointestinal abnormality. Despite the fact that the stomach still contained food, there was an increase in the heat production at one and two hours after ingestion. To us this factor of absorption is a large and uncertain one and must be carefully considered in all experiments dealing with the ingestion of materials by the gastrointestinal tract. Nor do we feel that the simpler substances we have used clears up this difficulty entirely, but that these substances would seem to be more readily and quickly absorbed hardly seems unreasonable.

To obviate this difficulty as much as possible, simple substances, sucrose and glycine were used in this study. Only four experiments were carried out with glycine since it was very unpleasant to ingest and fairly expensive. The amount of glycine used was in each case 50 grams and of sucrose 100 grams. To reduce the nausea which may be produced with glycine, it was administered in a large volume of fluid, 750 c.c., while with sucrose only 225 c.c. were used. The results of these experiments are summarized in Tables III and IV.

DISCUSSION

Although consistent results were obtained for determinations of basal metabolism throughout, in our control experiments in which the metabolism following the ingestion of sucrose was determined at periods of one-half, one hour, one and one-half and two hours, following its administration, we were unable to obtain consistent results with respect to either the magnitude or time of the maximal increase in heat production. The type of curves which we obtained are similar to those obtained by Benedict[14] but disappointing when compared to the consistent results which Lusk[15] obtained following the administration of glycine to dogs. With glycine we obtained only one control experiment.

Following the administration of the various pituitary preparations, the same irregularity of response to ingested sucrose was seen as was observed in the control periods. The response to glycine throughout was more consistent, and varied but little in the periods after pituitary substance was administered from the response in the control period.

The failure to demonstrate a regular quantitative metabolic response to the administration of these two relatively simple substances to a subject under ideal conditions casts doubts on the diagnostic value of the specific dynamic response to a protein meal as advocated by Plaut[11] and Liebesney.[13] Lauter[16] found a marked irregularity in the response of the metabolism of individuals who were fed a test meal of 200 grams of beefsteak and 50 grams of bread, and decided that the conclusions drawn from single three hour, or shorter determinations of specific dynamic action as used by Plaut, and also Liebesney cannot be considered as based on adequate evidence.

As far as can be ascertained from our data the administration of various pituitary substances influenced little the irregularity noted above.

It would seem therefore that before such a determination is available for diagnostic purposes, it is necessary to find a substance if possible which when administered will result in a definite even response of the metabolism of an individual and of a group of individuals.

SUMMARY AND CONCLUSIONS

1. Administration by mouth of whole pituitary substance (Wilson) and by intramuscular injection of antuitrin (Parke-Davis) and of pituitary solution obstetrical (Wilson) over comparatively long periods of time failed to influence the basal metabolism or the respiratory quotient in an apparently normal subject.

2. The increases in the metabolism after the administration of sucrose and glycine separately, were so irregular both with regard to time and magnitude of the maximal response both during the control periods and also following the administration of the above substances that conclusions regarding the effect of the administrations of pituitary substances upon the specific dynamic effect of food are unwarranted.

The authors wish to thank Prof. L. H. Newburgh, in whose laboratory this work was carried out, for his suggestions and interest in the problem; Dr. Franklin Johnston for assistance with the technical procedures and many other kindnesses; Miss Marie O'Connor for those determinations of basal metabolism done by the Benedict-Collins-Roth Method; and Miss E. Ackerman for the preparation of the diet.

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A STUDY OF THE OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF GENTIAN-VIOLET AND ACRIFLAVINE SOLUTIONS WITH REFERENCE TO THEIR STABILITY AND THERAPEUTIC VALUE*†

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THE intravenous use of gentian-violet and neutral acriflavine has given exceptionally variable and conflicting results in clinical and experimental work in recent years. Many of the factors supposedly contributory to these variations have been studied by various investigators, but the exact causes have never been satisfactorily explained. A fairly comprehensive view of the different phases on the subject may be obtained from papers of Kligler,[10] Brill and Meyers,[1] Young and Hill,[14] Reed and Lum,[12] Graham-Smith,[7] Burke and Newton,[4] Burke and Rodier,[5] Meleney and Zau,[11] Spencer,[13] and needs no elaboration here. Considerable attention has been given in these studies to the reaction of the dye solutions, to their toxic effect on body cells, to their bactericidal and bacteriostatic action in the presence of body fluids, and to their solubility in various solvents, but scarcely any work has been done on the osmotic pressure of these solutions.

A cursory examination of the various solvents recommended for the preparation of these dyes for intravenous injection suggests that there must be wide variations in the osmotic pressure of the solutions. Since the danger attending the use of hypotonic and hypertonic solutions for intravenous injections is generally recognized, it is possible that these variations affect the final results. The purpose of this study was to determine the osmotic pressure of some of the recommended solvents and dye solutions, and also to prepare solvents and dye solutions that are isotonic with the blood and have approximately the same reaction as the blood, thus making it possible to study their therapeutic value under better standardized and more carefully controlled conditions.

THE DETERMINATION OF OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF DYE SOLVENTS AND DYE SOLUTIONS

Several of the solvents generally recommended for the preparation of gentian-violet and neutral acriflavine and several of the recommended solutions of these dyes were prepared for osmotic pressure and P_H determinations. The salts used for the solvents throughout these studies were Baker's c.p. chemicals. As these contain perceptible amounts of impurities, the osmotic pressure and P_H values naturally vary somewhat from values that would be

*From the Division of Bacteriology Washington Agricultural Experiment Station.

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obtained from absolutely pure chemical compounds. The dyes were the gentian-violet (medicinal) and the neutral acriflavine (pro injectione) prepared by the National Aniline and Chemical Company, of New York. It was found that different lots of these dyes varied somewhat in P_H but did not vary perceptibly in osmotic pressure. The dyes used in all the experiments here described were from the same lot. The osmotic pressure and P_H determinations of the dye solutions were always made on fresh solutions which were prepared at a temperature of 40° to 45° C. It was observed that with certain solvents temperatures higher than 45° C. had a marked effect on the stability of the dye solutions. Osmotic pressure determinations were made by the indirect freezing point and Harris and Gortner's[9] formula to calculate the osmotic pressure in atmospheres. This formula is $\pi = 12.06\Delta - 0.021\Delta^2$ in which π is the osmotic pressure in atmospheres and Δ is the lowering of the freezing point in degrees centigrade. The P_H determinations were made by the potentiometer method, using the hydrogen electrode.

The results of the determinations of the series of solvents and solutions are given in Table I. They show that the osmotic pressure varies from less than 1 atmosphere in dye solutions prepared with distilled water to more than 12 atmospheres in dye solutions prepared with 3 per cent sodium bicarbonate. The former is greatly hypotonic and the latter highly hypertonic with human blood which has an osmotic pressure of approximately 6.7 atmospheres. These wide variations may influence the results obtained by the intravenous use of these solutions if they are introduced into the blood stream in considerable amounts. Hanzlik, et al.,[8] observed distinct symptomatic changes following the intravenous injection of 0.1 per cent hypertonic sodium chloride which were not noticeable following injections of 0.9 per cent isotonic sodium chloride. On the other hand, 50 per cent glucose and 3 per cent sodium bicarbonate solutions, both of which are greatly hypertonic, have been used intravenously without serious effects. This suggests that the nature of the substances as well as the osmotic pressure of the solution may be important in bringing about symptomatic changes in the blood.

The P_H of these various solvents and dye solutions also show marked differences. There is considerable experimental work claiming that the hy-

TABLE I

OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF SEVERAL SOLVENTS AND DYE SOLUTIONS

SOLVENTS	SOLVENT		AFTER ADDITION OF 1 PER CENT GENTIAN-VIOLET		AFTER ADDITION OF 1 PER CENT NEUTRAL ACRIFLAVINE	
	P_H	P*	P_H	P	P_H	P
Distilled water	6.82		5.24	0.54	6.76	0.93
0.85 per cent sodium chloride	7.23	6.09	5.41	6.62	6.65	6.62
3 per cent sodium bicarbonate†	8.72	12.18	8.33	2.30	8.12	14.72
Buffered distilled water (0.300 gm. KH_2PO_4 + 0.387 gm. K_2HPO_4 per 100 c.c.)	6.67	5.32	6.13	2.34	6.45	3.06
4 per cent glucose	6.91			5.45		5.72

*Osmotic pressure in atmospheres.

†Solution precipitated when dye was added to the solvent.

drogen-ion concentration of dye solutions is a potent factor in toxicity and bactericidal effect of the dye. This is especially emphasized in the studies of Browning et al,[2] Eggerth,[6] and Burke and Newton.[4] Thus both osmotic pressure and reaction may be important factors in intravenous medication. Since these factors are controllable, it would seem desirable to regulate them to closely approximate those of the blood. The following experiment was designed with that object in view.

THE PREPARATION OF GENTIAN-VIOLET AND NEUTRAL ACRIFLAVINE SOLUTIONS WITH APPROXIMATELY THE SAME OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION AS THAT OF THE BLOOD

In selecting solvents for dyes for intravenous injections it is important to use materials that are not injurious when injected into the blood stream. Furthermore, these materials should have the property of increasing the P_H of the dye solutions as most dyes used for intravenous injections are acid in reaction. With the possible exception of sodium bicarbonate, Hanzlik et al[5] found disodium phosphate to answer this purpose most satisfactorily. They injected a slightly hypertonic (1.1 per cent) solution and a distinctly hypotonic (5 per cent) solution into dogs with resulting beneficial changes in their blood. Since sodium bicarbonate is not suitable as a solvent for gentian-violet and neutral acriflavine for reasons explained below, the phosphate salts were selected. For convenience the tri, di, and monopotassium phosphates were used in proportions suitable to obtain the desired osmotic pressure and P_H. These salts are well-known for their buffer effect and should prove very effective in correcting the acidity of the dyes. This was true with neutral acriflavine but was not so easily accomplished with gentian-violet. It was found that gentian-violet also has a powerful buffer effect which makes any change in its reaction to the alkaline side somewhat difficult, especially when the point of neutrality is approached. The reaction of P_H 7.6 was found to be about the critical point when potassium phosphates were used as solvents. At any P_H above this point the gentian-violet solution became unstable and this resulted in a general breaking down of the solution, making it unreliable for experimental work. Sodium bicarbonate seemed to have a similar effect. When 1 per cent gentian-violet was freshly added to a 3 per cent solution of sodium bicarbonate and the P_H determined immediately the potentiometer readings constantly changed until a P_H of above 8 was reached. This usually required several hours and resulted in a general breaking down and precipitation of the solution, making it unfit for the purpose of this study.

The use of an isotonic solution of dipotassium phosphate alone did not correct the reaction of gentian-violet in 1 per cent concentration to the desired P_H, so it was necessary to make use of tripotassium phosphate which has a much greater alkaline reaction. By making repeated trials with various proportions of tripotassium phosphate and dipotassium phosphate a 1 per cent gentian-violet solution with an osmotic pressure of 6.48 atmospheres and a P_H of 7.64 was finally obtained. The solvent consisted of a mixture of 0.050 mols tripotassium phosphate and 0.050 mols dipotassium phosphate dissolved per

liter of freshly distilled water. When 1 per cent gentian-violet was added to this at 40° C. the resulting solution was relatively stable for twenty-four hours as shown by repeated osmotic pressure and P_H determinations during that time. Another satisfactory solvent, consisting of a mixture of 0.051 mols tripotassium phosphates and 0.052 mols sodium acetate per liter of distilled water, was obtained. This also made a stable isotonic, alkaline solution with 1 per cent gentian-violet.

As noted above the preparation of a 1 per cent isotonic neutral acriflavine solution with the desired P_H did not present any difficulties because the buffer action of neutral acriflavine did not interfere to any marked extent. A few of the various combinations of the salts used as solvents and their effects on osmotic pressure and P_H of the dye solutions are reported in Table II. Molar concentrations of the salts rather than percentages by weight are given for convenience.

The results show how easily the P_H of gentian-violet solutions is affected by small variations in the amount of buffer salts at the critical point of reaction for gentian-violet. This should serve to emphasize the necessity of observing careful precision in preparing solvents for dye solutions for intravenous medication. The experiment demonstrated the possibility of preparing 1 per cent gentian-violet or neutral acriflavine solutions that have the same osmotic pressure as the blood and have a P_H similar to that of the blood.

It seemed now desirable to determine the effect of these standardized solutions on the therapeutic value of these dyes. The following experiments were undertaken for that purpose:

ANIMAL EXPERIMENTS WITH GENTIAN-VIOLET

The fact that gentian-violet is distinctly more acid than neutral acriflavine and consequently is more apt to be administered intravenously as an acid solution would seem to indicate that the changes, if any, resulting from the standardization of osmotic pressure and P_H could best be demonstrated by using this dye. To demonstrate these changes it was decided to use large

TABLE II

OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF VARIOUS BUFFERED SOLVENTS AND BUFFERED DYE SOLUTIONS

MOLAR CONCENTRATION OF BUFFERS	SOLVENTS		AFTER ADDITION OF 1 PER CENT GENTIAN-VIOLET		AFTER ADDITION OF 1 PER CENT NEUTRAL ACRIFLAVINE	
	P_H	P†	P_H	P	P_H	P
0.065 M* K_2PO_4 + 0.030 M K_2HPO_4	10.37	6.37	8.83	6.73		
0.059 M K_2PO_4 + 0.030 M K_2HPO_4	10.34	6.04	8.68	6.37		
0.050 M K_2PO_4 + 0.050 M K_2HPO_4	10.23	6.31	7.64	6.48		
0.048 M K_2PO_4 + 0.052 M K_2HPO_4	10.18	6.19	6.51	6.61		
0.054 M K_2PO_4 + 0.046 M $NaC_2H_3O_2$	10.30	6.07	8.82	6.49		
0.051 M K_2PO_4 + 0.052 M $NaC_2H_3O_2$	10.21	6.13	7.52	6.44		
0.040 M K_2PO_4 + 0.070 M K_2HPO_4	8.81	6.20			8.63	7.19
0.12 M K_2HPO_4 + 0.02 M KH_2PO_4	7.29	6.18			7.10	6.98

*Gram-mols per liter.

†Osmotic pressure in atmospheres.

doses for intravenous injections. Since gentian-violet is known to possess greater bactericidal properties in alkaline solutions than in acid solutions, the P_H of the dye was adjusted slightly above that of the blood. The solvent consisted of a mixture of 0.050 mols tripotassium phosphate and 0.050 mols dipotassium phosphate dissolved in a liter of freshly distilled water. One per cent gentian-violet was added to a convenient amount of this solvent at a temperature of 40° C.; the solution was gently shaken for two or three minutes, and filtered immediately through filter paper. By making corrections for the dye remaining in the flask and in the filter, it was found that the filtered solution contained 0.86 per cent gentian-violet. This solution was made up fresh for each injection and was injected at body temperature in the marginal ear vein of rabbits. The osmotic pressure of the gentian-violet solution was 6.48 atmospheres and the P_H was 7.64

Experiment 1.—To determine the effect of the intravenous injections of large doses of gentian-violet on normal rabbits.

Two full-grown, normal rabbits weighing between 3000 and 4000 gm. and one half-grown rabbit weighing 1940 gm. received each 20 mg. per kilo of isotonic alkaline gentian-violet solution. The injections were made in the marginal ear vein very slowly, allowing fifteen to twenty minutes for each injection. One of the full-grown rabbits developed spasms a few minutes after injection and died. The other two did not seem to be affected and resumed eating grass as soon as they were returned to their cages. One of these was later used for further experimentation on dye tolerance.

The experiment demonstrated that 20 mg. per kilo of the isotonic alkaline gentian-violet was not unusually toxic as two out of three rabbits did not show any injurious effects. This gave reasonable assurance that the toxicity of the dye was not increased by the use of the isotonic alkaline solution and that large doses could be used in determining its therapeutic value. This was done in the following experiment:

Experiment 2.—To determine whether a large dose of isotonic alkaline gentian-violet in the blood of a rabbit is sufficient to check an infection with Staphylococcus aureus.

Two full-grown, normal rabbits were injected intravenously with 2 c.c. of a heavy suspension of a virulent twenty-four-hour culture of Staphylococcus aureus. Twenty-four hours later both animals showed severe symptoms to the same degree. At this time one of the rabbits received 17 mg. per kilo of isotonic, alkaline gentian-violet solution. This was given very slowly allowing twenty minutes for the injection. No symptoms followed the injection. There was some improvement four hours after the injection. The following day the control rabbit died while the treated animal showed marked improvement as it was breathing normally and taking food. The following two days the animal was eating normally but was losing weight rapidly. The rabbit died thirteen days after the injection of the dye. An autopsy showed typical lesions in the heart, kidneys, and liver. The experiment showed that 17 mg. per kilo of isotonic alkaline gentian-violet retarded death resulting from a Staphylococcus aureus infection but did not prevent death.

The experiment was repeated with two half-grown, normal rabbits. Instead of injecting 17 mg. per kilo of isotonic alkaline gentian-violet, 15 mg. per kilo were administered, allowing twenty minutes for injection. No violent symptoms developed following the injections, but the animal appeared listless and died three hours later. The control rabbit did not die until the third day after the injection with a 2 c.c. suspension of Staphylococcus aureus. Since it was possible that the young rabbit receiving the dye happened to be more susceptible than the average rabbit, the experiment was repeated once more.

Two half-grown, normal rabbits were injected with a 2 c.c. suspension of virulent Staphylococcus aureus and twenty-four hours later one received 15 mg. per kilo of dye very slowly as in the previous experiment. No violent symptoms appeared, but the rabbit did

not show any signs of improvement six hours later. Death followed eighteen hours after the administration of the dye. The control rabbit died the night following the third day after the injection with Staphylococcus aureus.

The results of these experiments showed that the half-grown rabbits with severe symptoms of Staphylococcus aureus infections were unable to tolerate 15 mg. per kilo of the isotonic, alkaline dye solution and suggested that the toxicity of the dye was not reduced materially by controlling its osmotic pressure and P_H. To test this point more definitely the following experiment was designed:

Experiment 3.—To determine the toxic effect of repeated injections of 15 mg. dye per kilo on a normal rabbit.

In Experiment 1 the rabbit that received 20 mg. of dye per kilo and showed no symptoms following the injection was kept for three months and selected for this experiment because the animal had proved to be tolerant to an excessive dose of isotonic, alkaline gentian-violet. At the end of three months the rabbit was still in perfectly normal condition and was now given 15 mg. per kilo of the isotonic, alkaline gentian-violet in the usual manner. When approximately 10 mg. of the dye were injected the rabbit showed signs of depression and the injection was discontinued immediately. Spasms developed suddenly and the animal died. Although the results of this single experiment are insufficient to draw definite conclusions, they seem to indicate that the tolerance of rabbits for gentian-violet is a function that is not constant for the individual, but one that may vary at different periods, although no differences in the physical appearance of the individual may be noticeable.

DISCUSSION

Many factors have been discussed in the literature in an attempt to explain the causes responsible for the conflicting results so generally obtained in intravenous dye therapy. Perhaps the hydrogen-ion concentration has received more attention than any single factor. Burke and Grieve[3] believed that the ultimate fate of the dyes as chemotherapeutic agents in injections is largely dependent upon the effect and control of the hydrogen-ion concentration. The results of the experiments reported here do not support this belief. The beneficial effects of gentian-violet solutions whose osmotic pressure was isotonic with the blood and whose hydrogen-ion concentration was slightly above that of the blood were not convincing. If it is assumed that the alkalinity of the blood is lowered in septicemia the intravenous injection of an isotonic solution with a powerful buffer effect, like this dye solution proved to possess, would be expected to be most favorable for positive results. The best that could be obtained here was temporary check of the progress of a Staphylococcus aureus infection retarding death for less than two weeks. It is doubtful that injections with smaller doses of dye would have given much better results. There is much experimental evidence indicating that gentian-violet in amounts of 7 to 11 mg. per kilo, administered intravenously, fails to show any appreciable bactericidal action and can at best only be expected to give bacteriostatic effects. Considering the existing danger attending the intravenous use of dyes it seems that it ought to be possible to demonstrate decided bactericidal action in animal experiments before they are advocated for general intravenous medication. Dye is eliminated from the blood very rapidly and has at best only a short time for its maximum activity.

Controlling the osmotic pressure and hydrogen-ion concentration to the most desirable point known failed to reduce the toxicity of gentian-violet solution to any marked degree. This would perhaps also hold true for neutral acriflavine. The work of Burke and Newton[4] and Burke and Rodier[5] shows that the toxicity of gentian-violet and neutral acriflavine did not vary very markedly although their solutions varied in hydrogen-ion concentration from P_H 5 to P_H 8 and in osmotic pressure from less than one atmosphere to more than 12 atmospheres. There is still the possibility that the solvent may play an important part in toxicity. Hanzlik et al[8] found that alkaline phosphates gave no serious reactions and were beneficial to the blood even when administered in slightly hypotonic and distinctly hypertonic solutions. The potassium phosphate solvents used here were isotonic and should cause no serious reactions intravenously. When mixed with one per cent gentian-violet no perceptible chemical change seemed to take place. A fairly stable solution resulted, and it would be expected that if the dye were nontoxic the intravenous use of the dye solution would cause no greater reactions than the intravenous use of the solvent. The experimental evidence of this study indicated that the dye solution was toxic and in one case caused death as a result of the intravenous injection of a moderate dose. It seems that the toxicity was not due to the osmotic pressure nor to the hydrogen-ion concentration, nor even to the solvent, but to the gentian-violet itself. The toxic effect did not seem to be a function that was constant for the individual but seemed to vary in degree from time to time within the individual. This would be expected if symptoms and reactions rest fundamentally on disturbances in important physical and chemical mechanisms of the blood and tissue as suggested by Hanzlik et al,[8] for minute but significant physical and chemical variations are bound to occur from time to time within the cells.

It seems that although osmotic pressure and hydrogen-ion concentration are important factors in intravenous dye therapy, toxicity and chemotherapeutic activity of gentian-violet, acriflavine, and dyes in general are strictly functions of the chemical constitution of the dye molecule and are controllable within limits by chemical manipulation. Therefore, future progress of intravenous dye therapy should have its greatest possibilities in the chemical manipulation of the dye molecule with a resulting increase in its chemotherapeutic activity and a decrease in its toxicity.

SUMMARY

1. Osmotic pressure and hydrogen-ion concentration determinations of several solvents and dye solutions used for intravenous injections showed wide differences in osmotic pressure and hydrogen-ion concentration.

2. Both the osmotic pressure and the hydrogen-ion concentration of gentian-violet and neutral acriflavine solutions may be controlled simultaneously to approximate those of the blood.

3. Gentian-violet was found to be a powerful buffer up to a point of about P_H 7.6. Above this point the solution was unstable and broke down rapidly when potassium salts and sodium bicarbonate were used as solvents.

4. A one per cent isotonic, alkaline gentian-violet solution did not seem to increase or decrease the toxicity of the dye to any appreciable extent when injected intravenously into the marginal ear vein of rabbits. The therapeutic value of the dye did not seem to be improved markedly by controlling the osmotic pressure and hydrogen-ion concentration to a point closely approximating those of the blood.

5. The toxicity of gentian-violet was found to be a function which is not constant within the individual but which may change in degree from time to time.

6. It is suggested that although osmotic pressure and hydrogen-ion concentration are important factors in dye therapy, the toxicity and chemotherapeutic activity of the dye are primarily functions of the chemical constitution of the dye molecule and can best be controlled by chemical manipulation of the molecule.

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INCREASING WEIGHT WITH INSULIN*

Preliminary Report

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RELATIVELY little is to be found in the literature of this country regarding the use of insulin in the treatment of individuals who for one cause or another are underweight. In 1924 Marriott[1] and Barbour[2] each published an article on the use of insulin in malnutrition of childhood. An article on the same subject was published by Tisdall et al.,[3] in 1925 and one by Fischer and Rogatz[4] in 1926. These all concerned the use of insulin in malnutrition of childhood. Careful search of the literature has not revealed any reports of its trial in adult malnutrition in this country, although many such references are to be found in the foreign literature and especially that of Germany and France. Such reports can be briefly summarized by stating that the results were almost universally successful in the attempt to force feeding and increase weight in nondiabetic malnutrition. It has been found especially valuable for building weight and aiding convalescence in pulmonary tuberculosis. Although it would be impossible in the brief scope of this article to relate the various experiences reported by foreign observers,[5] one or more references may be cited.

Vogt[6] believes with Falta[7] that insulin affects not only carbohydrate metabolism but also the general metabolism, and accelerates intestinal digestion and absorption; he has found it of value in forced alimentation. Schellong and Hufschmid[8] summarize the progress in this direction and give the experience of their own clinic. They believe the class of patient to benefit by this treatment is affected with constitutional leanness and small appetite. Their experience would indicate that a condition of equilibrium is reached toward the close of the second week, as apparently the subject becomes in part habituated or immunized against the further action of insulin. Morini and Bouessée[9] obtained rapid weight increase in patients with pulmonary tuberculosis. They believe it of real value in this condition.

From these references and my experience it would appear that insulin can occupy a place of first importance in the therapeutics applied against malnutrition generally. A brief tabulation and description of several cases[10] so treated follow. Although the series is not extensive, results have been quite definite in all, and enough experience has been gained to make it possible to offer a few suggestions in the use of insulin for weight-producing purposes.

REPORT OF CASES

Case 1.—G. F., female, aged twenty-one, admitted to medical service July 6, 1927. History of progressive loss of weight over long period with anorexia, but no definite localizing

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of pathology. The patient had been in Mt. Sinai Hospital from November 29, 1926, to January 15, 1927; discharged improved; diagnosis: inanition.

Signs suggesting consolidation of right apex, but no râles. X-ray showed slight general hypervascularization but otherwise negative. Verne's test for active tuberculosis negative. Sputum negative. Temperature normal or subnormal throughout, except from July 13 to August 3, when there was a slight P.M. rise. Erythrocytes 4,410,000, hemoglobin 14.5 gm. (88 per cent). Leucocytes 4,500, polynuclears 57 per cent, lymphocytes 40 per cent. Glucose tolerance test normal. Blood chemistry: urea nitrogen 9.8, sugar 72, cholesterol 137, chlorides 478. Diagnosis: malnutrition, chronic pulmonary tuberculosis, anxiety neurosis.

CASE 1

DATE 1927	WEIGHT	DIET	B.P.	BASAL METAB.	REMARKS
July 6	60	Regular		−42	Extreme weakness and emaciation
8		"			
9		"			
12		"		−43	
15	55½	"			
16	55½	High caloric with intermediate feedings			
22	56½	"			Thyroid extract gr. 3 daily begun
25	58	"			Ovarian extract gr. 5 added
27	57½	"			
29	58½	"	75/55		
Aug. 1	57	"			
2	55	"			
3	55	"			
5	58	"		+7	
12	61	"	80/56		
19		"			
22		"			Insulin U. 3 t.i.d. 15 mm. a.c. begun
26	66	"			
29		"			Insulin increased to U. 5 t.i.d. a.c.
Sept. 2	73	"			General condition much better. Out of bed and much more cheerful
9	79	"			
13		"	98/60		
16	85	"			
23	90	"			Discharged from hospital in very good physical condition. Later observation showed further improvement. Insulin discontinued on leaving hospital

CASE 2.—M. S., female, aged seventy-four. Pneumonia twenty-one months previously. Under treatment for mastoid disease since. Very poorly nourished. Initial weight 100 pounds. Permanent improvement in health after insulin. Ambulatory.

CASE 2

DATE 1927	WEIGHT	DIET	REMARKS
Oct. 25	100	Mixed	Began taking insulin U. 5 before breakfast and supper only
Nov. 2	102	Mixed	Dose increased to U. 8 b.i.d. Appetite increased, more food consumed
4			
11	104	Mixed	Insulin U. 8 b.i.d. continued
15	106	Mixed	Third week of insulin administration completed. Feeling much better and stronger.

CASE 3.—B. McC., male, aged twenty-six. Under observation for two years for general poor health; underweight, furuncles, Vincent's angina, etc. Small discharging sinus in gluteal region. No signs of pulmonary tuberculosis at any time. Weight in October, 1925 was 115¼ pounds. Ambulatory.

CASE 3

DATE 1927	WEIGHT	DIET	REMARKS
Oct. 17	115¾	Mixed	Insulin U. 6 t.i.d. ½ hr. a.c. prescribed
25	120¼	Mixed	Reported inordinate hunger following insulin, and ingestion of unusual amounts of food
Nov. 8	117¼	Mixed	No insulin previous three days and only irregular doses before that. Becoming irritated by repeated injections. 8 units b.i.d. before breakfast and supper ordered.
17	119¾	Mixed	8 units b.i.d. ½ hr. before breakfast and supper. P. 76, B.P. 96/66. Insulin discontinued. No permanent improvement in weight though general health better

CASE 4.—E. M., male, aged sixty-one. Secondary anemia and malnutrition following acute bronchitis. Approximately 55 pounds under average weight for age and height. Ambulatory.

CASE 4

DATE 1927	WEIGHT	DIET	ERYTHROCYTES	HEMO.*	REMARKS
Oct. 14	118½	Mixed regular	3,200,000	65	Rather feeble. Lungs clear. Insulin U. 5 t.i.d. a.c. prescribed
20	122	Mixed regular			Dose increased to U. 8 t.i.d. a.c.
28	123	Mixed regular			To continue U. 8 t.i.d. a.c.
Nov. 4	124½	Mixed regular	3,980,000	72	To continue U. 8 t.i.d. a.c.
11	125	Mixed regular	3,960,000	72	To continue U. 8 t.i.d. a.c.
17	127	Mixed regular	4,100,000	75	To continue U. 8 t.i.d. a.c.
25	127½	Mixed regular			To continue U. 8 t.i.d. a.c.
Dec. 2	127½	Mixed regular	4,380,000	78	Insulin discontinued this date. Only moderate stimulation of appetite by insulin during period
9	127	Mixed regular			No insulin past week

CASE 5.—M. K., female, aged seventy. Chronic "indigestion." Visceroptosis. Approximately 50 pounds under average weight for age and height. Ambulatory.

CASE 5

DATE 1927	WEIGHT	DIET	REMARKS
Oct. 25	110	Mixed	Insulin U. 5 b.i.d. before breakfast and supper prescribed
26	110	Mixed	Increased appetite noted
Nov. 2	108¾	Mixed	Dose increased to 8 units b.i.d.
4		Mixed	Eating more heartily
11	107½	Mixed	Three weeks of insulin completed. Patient says that she consumed much candy during period. Possible cause of failure
15	109¾	Mixed	

CASE 6.—G. C., female, aged forty-one, admitted to medical service September 23, 1927, discharged, improved, November 6, 1927. Diagnosis: visceroptosis, chronic pulmonary tuberculosis.

There was a regular afternoon rise in temperature of one-half degree during stay in hospital. X-ray showed old central tuberculous nodules with root branch thickening and general hypervascularization. Congestion was especially noted in right upper lobe. There was no definite x-ray evidence of recent activity. Verne's test for active tuberculosis was negative. Urine was essentially negative on repeated analysis. Fasting blood sugar on November 3 was 80 mg. Basal metabolism on September 29, 3 per cent below average normal. On September 24 erythrocytes numbered 4,296,000, leucocytes 8,500. Gastric analysis on September 26 was normal.

CASE 6

DATE 1927	WEIGHT	DIET	REMARKS
Sept. 23	86	Regular	Admission
27		Regular	Insulin begun. U. 5 at 8:30 A.M. and 4:30 P.M.
28		High caloric	
30	90	High caloric	
Oct. 4	90	High caloric	
6	90	High caloric	
11		High caloric	Insulin increased to U. 5 at 8 and 11 A.M., and 4 P.M.
13	91	High caloric	Insulin increased to U. 5 at 8 A.M., U. 8 at 11 A.M. and 4 P.M.
14	96	High caloric	
15-20	96	High caloric	Blood pressure on October 18, 115/65
21	97½	High caloric	
22	96½	High caloric	
24	96½	High caloric	
25	97½	High caloric	Insulin increased to U. 8 at 8 A.M., 11 A.M., and 4 P.M.
26	98½	High caloric	
27	100	High caloric	
28	101	High caloric	Blood pressure 130/80
29	100	High caloric	
31	100	High caloric	
Nov. 1	101	High caloric	
2	101	High caloric	
4	102	High caloric	
5	101½	High caloric	Insulin discontinued; patient discharged Nov. 6

CASE 7.—H. H., female, aged thirty-three. Average weight during adult years has been between 90 and 95 pounds and often as low as 88 pounds. She has been in fair health in the past except for a severe attack of septicemia lasting from November, 1925 to May, 1926. Recovery was complete and health has been as before. Easily fatigued. Appetite poor most of time. Eats very little breakfast, if any, and only small portions at other meals. Weight in December, 1927, 91 pounds.

This patient was started on 10 units of insulin b.i.d., half hour before breakfast and before supper. She was followed for only a few days as she was obliged to leave the city, and is mentioned in this article only because of the astonishing effect produced upon her appetite and attitude toward food. A typical breakfast after insulin was a large portion of oatmeal with cream, a half grapefruit, one fried egg, two thick slices of bread with butter, and a piece of cake. An enormous dinner was likewise consumed following the second dose at evening. It seems inevitable that this subject would have gained considerable weight if this routine had been continued.

COMMENT

The foregoing case reports indicate that insulin is without doubt of value in increasing weight in conditions other than diabetes; or, in other words, where it can be assumed that the pancreas is normal and there is no diminution of sugar tolerance. The results obtained in Case 1 are perhaps the most striking, as here the percentage gain was greatest and the control period the longest. Later experience has suggested that greater success might have been obtained in Cases 2, 4, and 5 if more attention had been given to proper food selection.

Whether the total glycogen of the body is increased or decreased by the administration of insulin to normals (the literature contains rather contradictory evidence on this point, the weight of evidence favoring a decrease) it nevertheless is true that an individual can through insulin be induced to eat and assimilate a large amount of food, much of which will later be deposited as fat. This is the fact of practical importance in the use of in-

sulin for weight production. It is essential to take fullest advantage of the hunger engendered by the insulin. For this purpose a food mixture in which there is a large proportion of fat would seem logical. As a relatively small amount of sugar is sufficient to antidote promptly a dose of insulin and thus inhibit the food craving, it would seem that, in the attempt to increase weight, a food combination low in sugar but of a high caloric value in relation to weight and bulk should be selected. It is probable that the failure to increase weight in Case 5 was the result of eating much candy during the insulin period.

The dietary suggested above is almost the direct antithesis of that advocated by Schellong and Hufschmid, who advocate the giving of fairly large amounts of bread and honey or sugar both before and after the insulin injection. It would seem that this procedure is failing to capitalize on the intense food craving to the greatest extent by inducing the subject to partake of the greatest possible amount of fat-producing foods. In the majority of underweight cases it is probably not the alteration of metabolism which is desired primarily, but rather the ingestion of a larger amount of food. Insulin accomplishes this purpose. It is very questionable if Case 7 would have consumed as great quantities of food following insulin if sugar had been permitted before and after.

Psychic aversion to food as frequently encountered in the psychasthenic can be most successfully treated by insulin. In Cases 1 and 7 there was produced a change from anorexia with almost complete indifference to food to a marked relish and avidity. Experience has indicated that the optimal time for eating after a dose of insulin is about thirty minutes, as this interval usually produces the hunger and intense food craving without other alarming symptoms associated with hypoglycemia. A patient should always be warned, however, of what these symptoms are and how to combat them, as in the treatment of diabetes. Although the dose in this series was never increased above ten units t.i.d. I do not see any objection to doing so provided there is little or no response to smaller doses and the patient is sufficiently guarded against hypoglycemic shock. A better result could possibly have been obtained in Case 4 had a larger dose been administered.

Little can be said at present of maintaining weight once it has been acquired. This, however, would seem to be a relatively simple and easy matter. A better habit of eating having been established during the insulin period, it can be expected that the individual will continue to ingest sufficient food to maintain the increase. If necessary, an occasional short course of insulin therapy may be given.

The effect of insulin on the normal metabolism has not been studied as thoroughly as on that of diabetes. In diabetes the glycogen store of the liver is known to be increased by it, as is also that of depancreatized dogs, when sugar is fed. Bailey[11] found no increase in the basal rate after insulin, although an increase in the respiratory quotient is commonly observed, indicating an increased oxidation of carbohydrate. MacLeod[12] states that there is an "undoubted decrease in total glycogen which occurs on injecting insulin into

normal animals'' and that there must remain a fraction of available carbohydrate which changes into other metabolites, as not all is accounted for by oxidation and glycogen formation. However this may be, fat is produced in malnutrition under the influence of insulin. This is due in part, no doubt, to increased food intake; perhaps also in part to a direct influence of the insulin on general metabolism.

SUMMARY

1. Of seven cases of malnutrition treated with insulin, all showed increased appetite and some had intense food craving following insulin; five showed definite gains in weight in response to insulin; one showed no gain whatever but was slightly under the original weight at the end of three weeks; one was not observed a sufficient length of time.

2. It is concluded that insulin can be a valuable agent for increasing weight in malnutrition.

3. Attempts should be made to increase the fatty as well as the starchy foods after the administration of insulin when malnutrition is treated.

4. Thirty minutes should elapse after insulin administration before food is taken if the optimum development of appetite is desired.

5. The insulin should be given three times a day before meals in doses of 10 units more or less according to individual indications.

Since this article was prepared an article[13] bearing on the same subject has appeared in the literature. The authors conclude that ''insulin treatment properly controlled is a valuable adjunct in the treatment of certain critical cases of undernutrition in psychotic patients.'' They obtained usually a marked increase of weight with insulin.

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10 West Forty-seventh Street.

THE ADAPTATION OF STANDARD ANTIGENS TO THE PRECIPITIN TEST FOR SYPHILIS, WITH COMPARATIVE RESULTS SHOWN BY THREE THOUSAND SERA*

By H. W. Butler, M.D., New Orleans

THE principles governing the reactions of the different precipitation tests are essentially the same, so that when a particular method of performing the test is desired, the antigen is modified in such a way as to be applicable to that particular technic.

In a recent article,[1] I reported on the comparative results obtained with the Kahn precipitation test for syphilis, showing an agreement of 95.8 per cent with the standard Wassermann reaction. By the use of the Meinicke technic on a series of 500 cases, the percentage of agreement was found to be 97.5 per cent. In addition to the close agreement with the Wassermann and to the simplicity of the performance of the test, the sharp demarcation between the negative and positive reactions makes the test valuable by this method. The use of an acidulated or alkalinized antigen containing a gum resin flocculation indicator, as proposed by Meinicke, possesses distinct advantages over other methods of performing the precipitation test. As horse hearts used by Meinicke are difficult to obtain in America, I wish to present methods of adapting our standard beef heart antigens for use with this test and to show a comparative series of the results obtained with such modifications.

RESULTS OBTAINED BY USING MEINICKE TECHNIC AND THE HORSE HEART ANTIGEN PREPARED BY MEINICKE

One c.c. of the horse heart antigen is measured into a twenty c.c. test tube. Ten c.c. of a 3 per cent saline solution is measured into a second similar tube. Both tubes are placed in the water-bath at 45° C. for five to ten minutes, after which the contents of the tubes are poured together twice. One c.c. of this suspension, while warm, is pipetted into a test tube into which has been previously placed 0.2 c.c. of blood serum, not inactivated. The tubes are shaken and allowed to stand at room temperature overnight. Reading: The strong positive sera appear crystal clear with the sediment at the bottom of the tube. The moderately positive sera appear hazy with a considerable amount of sediment at the bottom. The weakly positive sera are slightly cleared with a small amount of sediment at the bottom of the tube. The negatives appear as when first set up, "milky," with no sediment.

SPINAL FLUIDS, HORSE HEART ANTIGEN (MEINICKE)

One-half c.c. of the emulsion, prepared as for blood sera, is pipetted into a tube containing 0.5 c.c. of spinal fluid and allowed to stand overnight.

*From the Department of Medicine, Tulane University School of Medicine.
Received for publication, May 17, 1928.

Strongly positive spinal fluids will settle out in the same way as the blood sera, but the microscope is necessary in most cases. A characteristic clumped precipitate appears in the positives and the negatives show no such precipitate.

COMPARATIVE RESULTS ON BLOOD SERA USING THE KOLMER WASSERMANN ANTIGEN AND THE ORIGINAL MEINICKE ANTIGEN

	Wassermann	*Meinicke*
72	Strongly positive	Strongly positive
5	Negative	Strongly positive
3	Negative	Moderately positive
8	Moderately positive	Strongly positive
6	Strongly positive	Moderately positive
1	Weakly positive	Strongly positive
4	Weakly positive	Moderately positive
1	Moderately positive	Moderately positive
2	Moderately positive	Negative
1	Weakly positive	Weakly positive
1	Anticomplementary	Weakly positive
2	Anticomplementary	Moderately positive
436	Negative	Negative
542		

COMPARATIVE RESULTS ON SPINAL FLUIDS USING KOLMER WASSERMANN AND ORIGINAL MEINICKE

	Wassermann	*Meinicke*
11	Strongly positive	Strongly positive
3	Weakly positive	Weakly positive
2	Weakly positive	Moderately positive
2	Negative	Moderately positive (Negative globulin) (Cell count—1)
1	Weakly positive	Strongly positive
65	Negative	Negative
84		

KOLMER ANTIGEN

Kolmer's Wassermann antigen can be changed into a precipitation antigen by diluting and adding a 5 per cent tincture of tolu (made from the balsam) as an indicator.

Antigen: 5 c.c. Kolmer antigen
25 c.c. Alcohol, 95 per cent
10 c.c. Tincture of tolu, 5 per cent

Diluting saline: 100 c.c. distilled water
3 gm. sodium chloride
0.15 c.c. of 10 per cent sodium hydroxide

Some antigens require more alkali than others, depending upon the concentration of lipoidal substance. Enough antigen should be added to this formula to reach the point of saturation for that particular antigen, the object being to make the suspension as unstable as possible without producing a

precipitate in the negative sera. It should be added to the point just short of this amount. The more antigen that can be added, the more sensitive the reaction; and the more alkali that is added, the less sensitive it is. The correct amount of antigen is obtained by testing against negative sera. The amount ranges from 2 to 5 c.c. if the Kolmer[2] method of extraction is used. The alkali is necessary to render the ether extract portion of the antigen suitable for emulsification.

One c.c. of the antigen is measured into a 20 c.c. test tube, and 10 c.c. of the alkaline saline measured into a second 20 c.c. test tube. These are placed in the water-bath at 56° C. for five minutes. They are then poured together twice. One c.c. of this suspension is measured immediately, while still warm, into a test tube containing 0.2 c.c. of blood serum which has not been inactivated. The suspension precipitates when it becomes cold, unless serum is added.

Spinal Fluids.—A Purdy tube is filled to the three c.c. mark with spinal fluid. Two c.c. of a saturated solution of ammonium sulphate are added and well mixed. It is allowed to stand at room temperature for fifteen minutes and then centrifuged.[3] The supernatant fluid is pipetted off, and the globulin is taken up in 0.2 c.c. of negative serum. One-half c.c. of the suspension as used for blood sera is added.

RESULTS OBTAINED WITH THE KOLMER WASSERMANN ANTIGEN, USING IT IN BOTH TESTS

	Wassermann	*Precipitation*
43	Strongly positive	Strongly positive
11	Moderately positive	Moderately positive
3	Weakly positive	Moderately positive
2	Weakly positive	Negative
1	Negative	Moderately positive
6	Moderately positive	Strongly positive
1	Weakly positive	Moderately positive
6	Weakly positive	Weakly positive
2	Anticomplementary	Negative
2	Strongly positive	Negative
4	Anticomplementary	Strongly positive
489	Negative	Negative
570		

Beef Heart Extraction.—Fresh veal hearts, the size of a small fist, are selected and the aurieles and superficial fat removed. The muscle is ground in a sausage grinder and spread on paper and dried by an electric fan. After it is completely dried, it is reground and extracted with ether as follows: Four hundred c.c. of ether are used to each 100 gm. of dry ground heart and allowed to act for ten minutes, shaking frequently. The ether is filtered off and 300 c.c. are added three different times to the heart muscle and treated in the same manner. The heart muscle is now dried completely free from ether, and for each gram of muscle, 5 c.c. of 95 per cent alcohol are added and

maceration is allowed to continue for three days at room temperature, after which the alcohol is filtered off and made up to the original volume with 95 per cent alcohol.

Some of this extract is saturated with cholesterin (to 100 c.c. add 0.6 gm. of cholesterin).

Veal Heart Formula.—

Veal heart extract	15 c.c.
Veal heart extract, cholesterinized	15 c.c.
Alcohol, 95 per cent	15 c.c.
Tincture of tolu, 5 per cent	15 to 20 c.c.
Alcohol containing 1 per cent benzoic acid	1 c.c.

Tolu varies. Add enough to the antigen to produce sufficient opacity when diluted. Too much tolu will obscure the precipitation. One c.c. should require 0.5 c.c. of N/10 NaOH for neutralization. Phenolphthalein is used as an indicator. The P_H is 4.8.

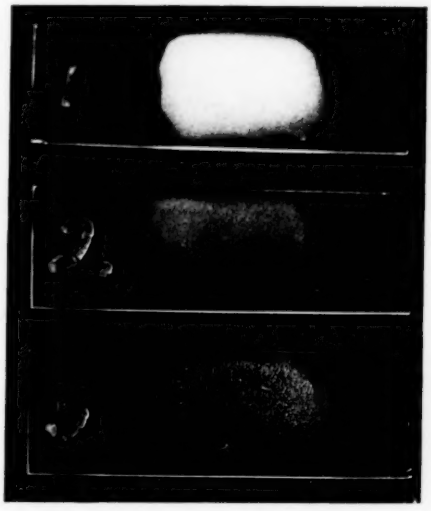

Fig. 1.—Butler slide method. No. 1, negative; No. 2, moderately positive; No. 3, strongly positive.

Formula.—

Veal heart extract	200. c.c.
Alcohol 95 per cent (containing 0.6 gm. cholesterin)	100 c.c.
Alcohol 95 per cent (containing 5 per cent of the balsam of tolu)	110 c.c.
Alcohol 95 per cent (containing 1 per cent benzoic acid)	5 c.c.

Mix and filter. This formula needs no adjusting.

Diluent.—3 per cent saline.

One c.c. of antigen is measured into a 20 c.c. test tube and 10 c.c. of 3 per cent saline into a second 20 c.c. test tube, heating in a water-bath as before at 56° C. two to five minutes. Heating at 56° C. for more than five minutes decreases

rapidly the sensitiveness of the test. Mix by pouring back and forth several times. One c.c. is measured into a test tube containing 0.2 c.c. of blood serum (not inactivated). Reading is made after standing overnight at room temperature.

Slide Test.—If the slide method is desired, the antigen is diluted only three times; i.e., 0.1 c.c. antigen and 0.3 c.c. of 3 per cent saline. A one c.c. pipette, as short as can be obtained, is fitted with a Wright's rubber bulb. The antigen is measured into a test tube with the dry pipette, and then, with the same pipette, 0.3 of 3 per cent saline is taken up and forced into the test tube containing the antigen. The mixture is sucked back and forth several times. No heating is required. Three drops of clear serum, using a capillary pipette, are placed upon a slide and three drops of the antigen mixture are placed alongside of the serum. It is mixed with a toothpick, and the slide rocked several times to insure thorough mixing. It is then laid aside for five or ten minutes, or longer, depending upon the amount of drying that takes place. As the preparation dries, it passes through different degrees of concentration, so that at some stage the zone of inhibition will be missed and precipitation allowed to take place. The positive bloods will show the characteristic precipitation, but the negatives will remain granular. The slide method should be performed at room temperature. It is slightly more sensitive than the tube method, and the reading is more striking.

Spinal Fluids.—The globulin is concentrated, using the Kahn technic[3] as for the Kolmer antigen. It is dissolved in 0.2 c.c. of normal saline or negative serum and one-half of a c.c. of suspension 1 to 10 is added. Strong positive spinal fluids can be read on a slide in the same way as a blood serum. Concentration is necessary in most cases, using 3 drops of concentrated globulin and 3 drops of the 1 to 3 suspension.

RESULTS WITH THE VEAL HEART ANTIGEN AND THE KOLMER WASSERMANN SYSTEM

	Wassermann	*Precipitation*
186	Strongly positive	Strongly positive
1	Strongly positive	Negative
1	Weakly positive	Negative
1	Negative	Strongly positive
4	Anticomplementary	Strongly positive
1	Negative	Moderately positive
9	Moderately positive	Strongly positive
10	Weakly positive	Strongly positive
10	Negative	Weakly positive
3	Weakly positive	Weakly positive
3	Strongly positive	Moderately positive
5	Moderately positive	Moderately positive
2	Strongly positive	Weakly positive
1776	Negative	Negative
2012		

Comments.—The Meinicke reaction, in its present form, is the nearest approach to the Wassermann in specificity of any of the precipitation tests in my experience. It is easy to perform: 0.2 c.c. of raw serum is measured into

a test tube, and 1 c.c. of the warm antigen suspension added. The tube is shaken and allowed to remain at room temperature overnight. It offers no difficulty in reading. If there is any criticism to make, it is that the horse heart extraction may be a little too sensitive. There is frequently a quantitative disagreement between the Wassermann and the precipitation reactions. A weakly positive Wassermann may give a strongly positive precipitation, and a strongly positive Wassermann may give a weakly positive precipitation. Occasionally, one reaction will be strongly positive and the other negative. Two Wassermann systems were used in checking the precipitin tests in many of these cases.

Conclusions.—The Meinicke precipitation technic, with some variations, can be applied to any of the standard antigens and the results obtained are commensurate with the sensitiveness of the antigen used. It is easy to perform and requires the minimum amount of time and laboratory facilities. Inactivation of the serum is unnecessary. There is no difficulty encountered in the interpretation of the results, which show a high degree of accuracy as compared with the Wassermann reaction.

NOTE.—This work was made possible by the excellent cooperation of Dr. G. H. Hauser and the entire staff of the Pathological Department of Charity Hospital, New Orleans, whose Wassermann system was checked.

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THE CONSTITUTION OF NEOARSPHENAMINE*

A New Structural Formula

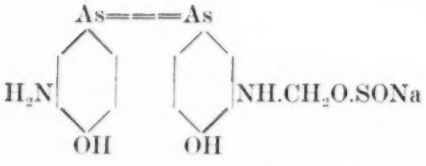
By Bernard Salkin, New York City

DURING an investigation on neoarsphenamine, a number of samples were examined by the method outlined by Macallum,† and it was found that if the calculations were made according to the method outlined in that article, the results were wrong on their face.

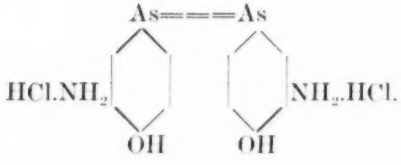
It was found that the arsenic in neoarsphenamine requires only *two* iodines for oxidation while the arsenic in arsphenamine actually requires *four* iodines for its oxidation. This led to a further investigation of the constitution of the compound, and the following results were arrived at:

1. There seems to be a regulation between the "sulphoxalate content" of the neoarsphenamine and the toxicity of the product;

2. The constitutional formula for neoarsphenamine; namely,



which was probably arranged



by analogy to arsphenamine,

and which is consistently found in the literature, does not express the facts;

3. The arsenic in neoarsphenamine is linked in a way that its oxidation from the tri- to the pentavalent state is "divalent," while the oxidation of the arsenic in arsphenamine (from the tri- to the pentavalent state) is "tetravalent."

The method used for the examination of the samples was that outlined by Macallum (cited above) and is essentially as follows:

Two grams of neoarsphenamine are dissolved in 100 mil. of water.

a. Ten mil. of this solution was acidified, and N/10 iodine solution added immediately. The excess was titrated back after three minutes with N/10 sodium thiosulphate solution.

b. Twenty mil. of the same solution was acidified. The free "sulphinic acid" (neoarsphenamine "acid") was filtered off, and to an aliquot portion of the filtrate, N/10 iodine solution was added. After three minutes the excess was titrated back with N/10 thiosulphate solution.

*From the Research Laboratories, Travis Colloid Research Company, N. Y.

†Jour. Am. Chem. Soc., 1920, xliii, 643.

c. The arsenic content was determined in 10 mil. of the solution.

Notes:

1. The results are all reduced to a basis of *mil of N/10 iodine per gram* of neoarsphenamine.

2. Calculations were made by the slide-rule.

The atomic weight for arsenic was taken as 75; the molecular weight of neoarsphenamine as 466; and the molecular weight of sodium formaldehyde sulphoxalate as 118.

The calculations were based on the following equations:

In arsphenamine	(AsIII)	+ 4I	= (AsV)
In neoarsphenamine	(As)	+ 2I	= (AsV)
In acid solution	$NaHSO_2CH_2O + 4I + 2H_2O$		$= NaHSO_4 + CH_2O + 4HI$

3. All flasks were filled with nitrogen gas and the solution in (b) was filtered in a nitrogen atmosphere.

4. The oxidation of arsenic to the pentavalent state is not complete in three minutes and this is reflected in the "combined sulphoxalate" result, giving a slightly higher result for the latter.

The result obtained from (a) is the number of mil. of N/10 iodine required for one gram of neoarsphenamine (total reducing power).

The result obtained from (b) is the number of mil. of N/10 iodine required for the "Free Reducing Substances" in one gram of neoarsphenamine.

From (c) we obtained the number of mil. of N/10 iodine required to oxidize the arsenic in one gram of sample.

Adding (b) plus (c) and subtracting the sum from (a), we obtain the number of mil. of N/10 iodine required to oxidize the sulphoxalate in the precipitated "Neo Acid," in other words, for the "combined sulphoxalate," (d).

The results are given in Table I.

If we use the calculations based on the "tetravalent" oxidation of the arsenic, we obtain the following results:

SAMPLE NO.	THEORETICAL COMBINED SULPHOXALATE ON THE BASIS OF ARSENIC CONTENT IN PER CENT	COMBINED SULPHOXALATE (AS CALCULATED) IN PER CENT
46	15.98	0.93
48	16.70	2.70
49	16.98	3.48
51	17.32	3.51
52	15.93	4.84
53	16.41	5.14
54	14.41	7.21
55	16.40	3.88
A	15.29	4.65
B	13.09	2.89

We must, therefore, conclude that there is a difference in the way the arsenic in neoarsphenamine oxidizes, from the oxidation of the arsenic in arsphenamine. Since in both cases the arsenic is oxidized to the pentavalent

TABLE I

NEO NO.	C.C. N/10 IODINE SOL. A	B	AS (75) PER CENT	C.C. N/10 IODINE SOL. C	D	COMBINED SULPHOXALATE (118) PER CENT	THEORETICAL COMBINED SULPHOXALATE ON BASIS OF ARSENIC CONTENT PER CENT	EXCESS COMBINED SULPHOXALATE OVER THEORY PER CENT
46	149.2	37.55	20.36	54.20	57.45	16.92	15.98	0.94
48	157.0	34.65	21.22	56.70	65.65	19.38	16.70	2.68
49	173.0	46.10	21.60	57.55	69.35	20.41	16.98	3.43
51	183.0	53.50	22.02	58.80	70.70	20.82	17.32	3.50
*52	174.5	50.00	20.30	54.10	70.40	20.75	15.93	4.82
53	178.6	49.70	20.92	55.75	73.15	21.60	16.41	5.19
*54	172.9	50.45	18.38	49.00	73.45	21.61	14.41	7.20
55	179.0	54.45	20.90	55.75	68.80	20.28	16.40	3.88
*A	159.0	39.30	19.45	51.90	67.80	20.00	15.29	4.71
B	132.0	33.40	16.65	44.45	54.15	15.99	13.09	2.90
‡	158.2		30.30	161.50†				

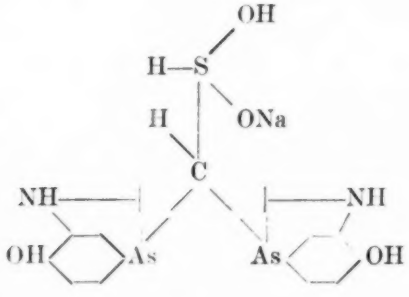
*Very toxic.
*A "Neo" precipitated by methyl alcohol.
B "Neo" made by a different process.
†The oxidation of As is not complete within three minutes.
‡Arsphenamine.

state, we must conclude that, whereas, in arsphenamine the two arsenic atoms are linked by a double bond, in the case of neoarsphenamine, the arsenic atoms are either bridged by a group which allows a normal oxidation to take place or they are not linked at all.

We thus come to the consideration of a new constitutional formula for neoarsphenamine.

While we have experimental proof that the formula is *not* as at present accepted, the new formula suggested is more in the nature of a hypothesis, and for the stimulation of further research.

Because of the results obtained by oxidation, we may assume that the following is the case:



Closing of the amine groups may be assumed from the fact that on precipitating neoarsphenamine with hydrochloric acid, no hydrochloride of the amine is formed. *The precipitate contains no chlorides.* This is directly opposed to the case of arsphenamine where two HCl groups are taken up before a neutral product is obtained. Another interesting observation made on the "sulphinic acid" is, that whatever be the composition of the original neo-

arsphenamine (as long as it is a monosulphoxalate) the precipitated "neo acid" will contain the theoretic proportions of carbon, hydrogen, sulphur, and arsenic.

Another explanation for neoarsphenamine might be that the sulphoxalate becomes an addition product of the arsenic (Cf. silver arsphenamine).

A third possibility is that, where the sulphoxalate is attached to one arsenic atom only, and the internal ring is closed through the NH_2 groups.

It is hard to believe that the present formula has been accepted in view of the three outstanding facts which oppose such a formula:

1. The fact that even a large excess of HCl does not precipitate a "hydrochloride."

2. The "divalent" oxidation is opposed to a double linking of the two arsenic atoms.

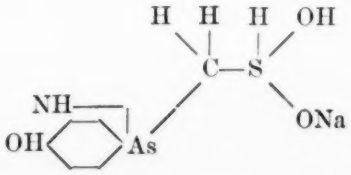
3. It is hardly conceivable that, whereas neoarsphenamine, the "sulphinic acid" of which contains the theoretic amount of sulphoxalate to form the "monosulphoxalate" is nontoxic; the additional content of sulphoxalate, which is itself not toxic, causes toxicity of a high degree. As the sulphoxalate is not toxic, its further addition to arsphenamine in a position similar to that in which the first group had been added, the amine group, should not form a toxic product.

This brings us to a consideration of toxicity.

The sample, No. 46, was the only one of the group examined which was not toxic. In this sample the relation of sulphoxalate to arsenic is nearly theoretical. (It is probably closer to theory than the result shows.)

In the case of the other samples, and they were all toxic, there is more sulphoxalate than the theory calls for in a mono-substitution product. This means that there is actually present five times as much di-substitution product as the percentage of excess sulphoxalate. For example, sample No. 53, analyzing 5.19 per cent excess sulphoxalate, probably contains 25 per cent of the di-substitution product, and thus would be very toxic.

If we assume the new formula for neoarsphenamine, we can understand the reason for a toxic compound being formed in the case of the di-substitution product. As soon as the second sulphoxalate group gets into the molecule, the arsenic atoms are no longer linked with one another, but break apart, each one probably having a sulphoxalate group attached to it, or what is even more probable, the arsphenamine molecule is broken into two similar molecules, each having only one benzene ring; e.g.,



SUMMARY

1. The relation of combined sulphoxalate to arsenic (in the so-called "sulphinic acid" portion of neoarsphenamine) can be found by oxidizing the neoarsphenamine in steps. From the results obtained we can tell whether the product will be toxic or nontoxic. The nearer the ratio of combined sulphoxalate to arsenic is to theory, the less toxic will be the product.

2. The structure of the molecule of neoarsphenamine, as it is at present accepted does not express the real state of affairs. The new formula suggested, where the sulphoxalate group is attached directly to the arsenic and not to the amine group, is much more in agreement with experimental results.

3. The new facts presented should be an aid in the manufacture of a neoarsphenamine of low toxicity but high therapeutic value.

HEMOAGGLUTINATION

III. Hemoagglutination in the Blood of Chickens*

By Warner M. Karshner, B.S., M.D., F.A.C.S., Seattle

IN FORMER papers of this series a brief statement was made covering work that has been done on hemoagglutination in the blood of animals. It is unnecessary to repeat that outline here. The technic therein described has been followed in the main and only new features will be noted in this paper.

TECHNIC

I. The chicken blood was secured from two widely different sources, namely: (1) Meat shops where the birds were prepared for retail trade, and (2) from the thoroughbred White Leghorn strain of the Western Washington Experiment Station.

II. The blood secured from public markets was often grossly contaminated, due, no doubt, to the nature of surroundings and the method of collection. It did not preserve well, even in the frigidaire; moreover, it was necessary to use 2 per cent sodium citrate normal salt solution to prevent coagulation. This necessitated repeated washings in normal salt solution to bring the cells to isotonicity.

ISOAGGLUTINATION

Table I shows a series of sixty-four cross-typings of chicken blood. Of these sixty-four double typings, five only showed positive at both ends of the slide; fourteen showed positive at one end; forty-five double tests proved negative. In other words, of the one hundred twenty-eight separate tests, but twenty-four showed agglutination.

*From the Bacteriology Department, University of Washington, Seattle.
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TABLE I

RESULTS OF CROSS-AGGLUTINATION OF CHICKEN RED CELLS WITH CHICKEN SERUM

SERUM X. O R. C. Y.		SERUM Y. O R. C. X.
	NO. CASES	PER CENT
Both positive	5	8
One positive	14	22
Neither positive	45	70
Total	64	100

We note the absence of reactions that might be expected if the blood types of chickens were well developed and in comparatively large groupings. The fact that more agglutinations do not occur can be explained in two ways; namely, either the types in chickens are not clear and well developed, or the subnormal types are of relatively small percentage. The latter view, it would seem, harmonizes better with the known facts, since agglutination, when it does occur, usually appears prompt, vigorous and typical. Iso-agglutination, moreover, as observed in the above series, appeared far less common among the thoroughbred White Leghorn flock than among the mixed strains and mongrels common in the open market. High percentages of positive agglutinations were also found when mixtures from these two sources were typed.

From a study of Table I it appears obvious that there are at least three distinct blood groups in chickens:

Group A.—This constitutes by far the largest group. The red cells show no agglutination when mixed with serum from groups B or C.

Group B.—This is a much smaller group. The red cells agglutinate when mixed with serum from Groups A and C.

Group C.—This is the smallest group. The erythrocytes are not only clumped by the sera of Groups A and B, but it also invokes agglutination in the red cells of Group B.

It will be seen that Groups B and C are wholly incompatible, i.e., the sera and red cells mutually agglutinate each other.

HETEROAGGLUTINATION

Heteroagglutination was defined in Parts I and II of this series of papers. Its presence in chicken blood is shown in a startling way in the following tables:

TABLE II

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH BOVINE SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
100	100	0	100	0

In this series of one hundred cases, the red cells of chicken blood were mixed with bovine serum. In not a single instance did heteroagglutination fail to appear promptly.

TABLE III

RESULTS OF AGGLUTINATION OF BOVINE RED CELLS WITH CHICKEN SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
100	100	0	100	0

This table shows one hundred positive reactions when bovine red cells were mixed with chicken serum.

TABLE IV

RESULTS OF AGGLUTINATION OF ADULT HUMAN RED CELLS WITH CHICKEN SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
100	100	0	100	0

This shows one hundred cases where adult human red cells were mixed with chicken serum. In every instance agglutination occurred. The cells were clumped into large compact masses, larger and more firmly adherent than obtained when other sera were employed.

TABLE V

RESULTS OF AGGLUTINATION OF HUMAN UMBILICAL RED CELLS WITH CHICKEN SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
101	101	0	100	0

This result is similar to the one in Table IV, except that the red cells came from the umbilical cord following delivery. In one hundred cases the erythrocytes gave a positive reaction. So far as could be observed, they were fully matured. This agrees with the findings in Part I, "Isoagglutination in the Blood of Infants."

TABLE VI

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH HUMAN UMBILICAL SERUM

SERUM TYPES	NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
II	12	6	6	50	50
IV	12	6	6	50	50

In this limited series chicken red cells were mixed with Types II and IV sera from human umbilical blood. Each type was represented by two different specimens, showing six tests, respectively. It will be seen that each specimen reacted consistently; when it caused agglutination in one instance, it did so in every instance; when it failed to react in one, it failed throughout the tests. These findings naturally suggest that the sera in the negative tests were immature and led to the following typings:

TABLE VII

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH TYPES II AND III SERA FROM ADULT HUMAN BLOOD

SERUM TYPES	NO. CASES	AGGLUTINATION +	AGGLUTINATION −	PER CENT +	PER CENT −
II	104	0	104	0	100
III	104	88	16	85	15

Heteroagglutination, as shown by Table VII, is indeed surprising. It shows 85 per cent positive reactions between Type III adult human serum and chicken erythrocytes, while Type II human serum when mixed with the chicken red cells did not show a single positive reaction in the whole series of 104 cases! This failure to react cannot be charged to defective Type II stock serum, since two separate allotments of sera were employed; moreover, they were simultaneously used with good results in typing both bovine and human umbilical bloods. Certainly, both allotments of Type II serum lacked the proper agglutinin necessary to clump chicken red cells but did not possess it for both human umbilical and bovine cells. Inquiry revealed the fact that both stocks of Type II sera used above, were supplied by the same donor. It was assumed, therefore, that it was an individual characteristic and, to definitely ascertain the truth, a large assortment of fresh human sera were secured.

TABLE VIII

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH TYPES II, III, AND IV ADULT HUMAN SERA

		CHICKEN RED CELLS							
HUMAN SERA	TYPES	1	2	3	4	5	6	7	8
A.	II	–	–	–	–	–	–	–	–
B.	III	+	+	+	+	+	+	+	+
C.	II	–	–	–	–	–	–	–	–
D.	II	–	–	–	–	–	–	–	–
E.	II	–	–	–	–	–	–	–	–
F.	II	–	–	–	–	–	–	–	–
G.	II	–	–	–	–	–	–	–	–
H.	II	–	–	–	–	–	–	–	–
I.	II	+	+	+	+	+	+	+	+
J.	IV	–	–	–	–	–	–	–	–
K.	IV	+	+	+	+	+	+	+	+
L.	IV	+	+	+	+	+	+	+	+

In this series, chicken red cells were mixed with twelve different human sera. A. and B. were the stock sera shown in the reactions of Table VII, and used here as controls. Of the ten remaining sera, seven gave negative results only. One Type II and two Type IV, gave only positive reactions. These results, condensed and retabulated, are shown in Table IX.

TABLE IX

RESULTS FROM TABLE VIII, CONDENSED AND RE-TABULATED

SERUM TYPE	AGGLUTINATION +	AGGLUTINATION –	TOTAL	PER CENT +	PER CENT –
II	8	56	65	12.5	87.5
III	8	0	8	100	0
IV	16	8	24	66.2/3	33.1/3
Total	32	64	96	33.1/3	66.2/3

It will be seen from a study of this table that heteroagglutination resulting from a mixture of chicken red cells and human serum, is not a constant phenomenon. In fact, it is far less common than that obtained by mixing chicken red cells and bovine serum. Repeated washings of the chicken red cells in normal salt solution to remove all traces of chicken serum, did not alter the findings; neither were the results materially affected by recourse to fresh unheated sera.

CONCLUSIONS

1. Isoagglutination occurs occasionally in the blood of chickens.

2. It appears more common among those of different breeds than among those of the same or similar strains.

3. At least three types can be distinguished: Type A, the largest group, that does not appear reactive. Types B and C, two smaller groups, which show agglutination when the red cells are separately mixed with serum A. These groups are incompatible, i.e., when cross-typed they will agglutinate each other.

4. Heteroagglutination resulting from a mixture of chicken and bovine bloods appears a constant phenomenon.

5. Heteroagglutination resulting from a mixture of chicken serum and human red cells appears constant; with the blood elements inversely mixed, it often fails to appear.

NOTE: For references see Hemoagglutination in the Blood, II, JOUR. LAB. AND CLIN. MED., December, 1928.

STUDIES IN TOXICOLOGIC CHEMISTRY*

III. THE REACTIONS OF THE PHENOLS AND PHENOLIC OR OPIUM ALKALOIDS WITH MARQUIS' REAGENT, THE NITRIC-ACID-FORMALDEHYDE REAGENTS, AND ERDMANN'S REAGENT

BY VICTOR E. LEVINE AND CHARLES C. FULTON, OMAHA, NEBRASKA

INTRODUCTION

IN THE first article of this series Levine[1] showed that the phenol group is responsible for the reaction given by the opium alkaloids with Lafon's or Mecke's reagent, a solution of selenious acid in concentrated sulphuric acid. In the second article Levine and Magiera[2] showed how the tests for morphine should be classified. It was also shown in the latter article that the morphine reaction with Marquis' reagent (formaldehyde in concentrated sulphuric acid) belongs to the group of tests depending on the presence of the phenol group.

Since then Fulton[3] in an independent article has announced two new reagents for the opium alkaloids. These were called "Nitric-acid Formaldehyde Reagents," both being made from nitric acid, formaldehyde, and sulphuric acid. One reagent differs from the other by dilution of its components. They were distinguished as Reagents A and B. To avoid unduly cumbersome names they will hereafter be referred to as the Fulton reagents.

It seemed highly probable that these new reagents also react with the opium alkaloids because of the phenolic character of the latter. If this were the case, it would be interesting to find certain nonalkaloidal phenols giving the same reaction as morphine or its allied compounds, or yielding characteristic reactions useful in identification. With the foregoing ideas in mind, this present study was instituted.

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It seemed best, in this connection, to repeat the tests with the Marquis reagent. In the previous study the time during which the reaction mixture was kept under observation was indefinite, and extended in some cases to several days. In this study we have limited our observations to a period of about fifteen minutes. Laboratory air generally contains reactive substances, so that changes occurring very slowly or only after long standing may be of doubtful significance.

A survey of the reactions of Erdmann's reagent with opium alkaloids and phenols has also been included. This reagent, sulphuric acid containing a small quantity of nitric acid, was originally introduced as a test for the opium alkaloids. We have found it to react with the phenols in general.

THE REAGENTS

The reagents investigated were made in the exact manner described:

FORMALDEHYDE-SULPHURIC-ACID REAGENT (MARQUIS' REAGENT)

Two drops of U.S.P. formaldehyde solution (37 to 40 per cent) were added to 3 c.c. of concentrated sulphuric acid. The reagent deteriorates and should be prepared on the day used. Tested on brucine it should give no color.

THE NITRIC-ACID-SULPHURIC-ACID REAGENT (ERDMANN'S REAGENT)

Four drops of concentrated nitric acid were mixed with 100 c.c. of distilled water. Five drops of this solution were added to 5 c.c. of pure concentrated sulphuric acid to form the Erdmann reagent.

NITRIC-ACID-FORMALDEHYDE-SULPHURIC-ACID REAGENTS (FULTON'S REAGENTS)

Reagent A. To one drop of concentrated nitric acid in a clean, dry test tube were added four or five drops of U.S.P. formaldehyde (37 to 40 per cent) and 5 c.c. of pure concentrated sulphuric acid. A violent reaction takes place on the addition of the sulphuric acid. When the effervescence subsides the reagent is ready for use. The reagent should be freshly prepared. If used throughout the day, it becomes necessary to test it at intervals on known samples of morphine and codeine.

Reagent B. One drop of concentrated nitric acid in a clean, dry test tube was mixed with three drops of U.S.P. formaldehyde (37 to 40 per cent) and 1 c.c. of pure concentrated sulphuric acid. One c.c. of water and 4 c.c. of concentrated sulphuric acid were now added, and the mixture cooled by means of running water. The reagent should give the following reactions with known samples of morphine and codeine.

Morphine—brown (without any purple, green, or blue) changing gradually to red.

Codeine—olive, soon developing to deep bright blue, and gradually developing purple, at least in the center of the solution.

The heat of mixing or the proportion of nitric acid to formaldehyde may have to be varied in order to produce a satisfactory reagent.

Reagent A is made with the components of the Marquis and of the Erdmann reagent. The nitric acid and the formaldehyde react violently, and the

formaldehyde is in excess. The reagent is therefore more nearly like Marquis' reagent than Erdmann's. However, the nitric acid does not lose the properties which it displays in Erdmann's reagent.

Since Reagent B is made from Reagent A by the addition of water, its characteristic reactions must be due to the same cause. It has the formaldehyde only very slightly in excess of the nitric acid. With some compounds it reacts much like a very sensitive Erdmann reagent. This is the case with morphine and codeine. Reagents A and B may be regarded as links connecting the Marquis and Erdmann reagents.

The Marquis reagent and the nitric-acid formaldehyde reagents are quite sensitive to phenols, and very small amounts of the latter suffice to give strong reactions. Erdmann's reagent is less sensitive, and larger amounts of phenols have to be employed in testing.

DESCRIPTIONS OF THE COLOR REACTIONS

It should be remembered that two people seldom describe the same color reaction in the same way. In making use of any reactions for the identification of an unknown, the analyst should always first try, if possible, the effect of the reagent on a known sample of the suspected substance.

In speaking of the various reactions we shall in the course of the paper refer to some of them as distinctive, characteristic, or specific. For the sake of clearness we shall define these terms in the sense that we employ them. By a *distinctive reaction* is meant one in which clear, bright, strong, and easily recognizable colors are obtained. By a *characteristic reaction* is meant one in which an easily recognizable color or succession of colors is obtained, and which is given by few, if any, other compounds which might be confused with the one for which the test is made. By a *specific reaction* is meant one given only by the compound for which the test is made and by no other substance so far as known.

REACTIONS OF THE OPIUM ALKALOIDS

The four reagents used were all introduced as giving useful color reactions for the identification of the opium alkaloids. Marquis' and Erdmann's reagents have been known for many years, but the nitric-acid formaldehyde reagents were introduced only last year. These reagents all react with the phenols in general.

The reactions with the opium alkaloids are given in Table I.

Erdmann's reagent is not of any great value.

The three reagents containing formaldehyde give distinctive reactions with morphine, codeine, oxydimorphine, apomorphine, papaverine, and narcotine. These alkaloids are readily distinguished from each other by means of these reactions. We shall later discuss the possibility of confusing them with other phenolic compounds.

With the four reagents heroine reacts like morphine and dionine like codeine.

REACTIONS OF THE PHENOLIC COMPOUNDS WITH THE FOUR REAGENTS

In Table II we report the detailed events following the application of the four reagents tested upon monophenols and their derivatives, diphenols

TABLE I

ALKALOIDAL REACTIONS WITH THE FOUR TESTED REAGENTS

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Morphine and Heroine	crimson, gradually changing to purple then slowly to blue	momentary purple changing to green, then to blue, then to dirty violet-red then slowly to green	brown changing to red	brown with tinge of orange
Codeine and Dionine	purple changing to blue-purple	green changing to very dark green then slowly to dark blue, then slowly to brown	olive, quickly changing to deep bright blue then gradually to purple-blue	light yellow changing quickly to green then slowly to blue
Oxydimorphine	orange-red changing to deep bright red	olive green changing to strong bright blue-green	brown slowly changing to light blue-green then to green	yellowish changing to dull green
Apomorphine	purple quickly changing to dark blue then gradually to dark green	purple changing to dull dark green, then slowly brownish-red-purple develops	dirty green changing to blue then slowly to deep purple	dirty dull green changing gradually to deep red-violet
Papaverine[1]	red-purple develops slowly changing to red then slowly to brown-red	deep bright blue, soon develops tinge of green at center, changing to deep blue throughout	light gray gradually changing to deep bright blue	negative
Narcotine[2]	purple soon changing to dirty brown, gradually changing to yellow, slowly to brown	dirty violet-red changing to brown then to bright red	yellowish changing to bright carmine	salmon changing to orange-yellow then gradually to orange, then slowly to red
Narceine[3]	orange develops changing to red then to brownish-red	orange develops changing to red then to brown	yellowish-brown with tint of green; then brownish solution, purplish at edge; changing to brown-orange. The colors are poor	yellowish-brown with green tint
Thebaine	Concentrated sulphuric acid alone, and the reagents, give brown-red, deep red-orange on solution.			
Cotarnine hydrochloride	Concentrated sulphuric acid, and the formaldehyde-reagents, give light yellow.			weak orange changing to orangish-yellow at once

[1]In concentrated sulphuric acid alone the solid assumes a purple color and dissolves with a slight purple color.

[2]In concentrated sulphuric acid alone a yellow color is formed possessing a faint tinge of green.

[3]Concentrated sulphuric acid alone gives yellowish-brown, on solution brownish-yellow, gradually becoming somewhat orange.

TABLE II

REACTIONS OF THE FOUR TESTED REAGENTS WITH PHENOLIC COMPOUNDS

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Phenol	momentary purple-red changing to deep red insoluble, sticky mass	red-brown changing to dark brown then to deep red	brown-orange changing to dark brown then to dark green then slowly to dark red	light orange or yellow changing to light green then to light blue; slowly fades out
o-Cresol (methyl phenol)	intense red, at first scarlet, later purplish	dark brown changing to red	brown changing to dark green, then gradually to dark red, beginning at the edge	yellow changing to light orange
m-Cresol	momentary purple-red changing to deep red insoluble, sticky mass	dark brown changing to deep scarlet	orange-brown changing to dark brown then to deep red	yellow changing to green; slowly fades out
p-Cresol	dark brown, insoluble	dark brown changing to olive-brown	dark brown changing slowly to dark red	brown or orange-brown
o-Xylenol (4) (dimethyl phenol)	dark brown	dark brown	brown changing slowly to dark red	slight brown-orange, changing to pink
m-Xylenol (2)	orange changing to deep red	strong brown quickly changing to deep red	dark brown soon changing to dark red	brownish-yellow changing to brown
m-Xylenol (4)	brownish-yellow changing to dark brown	dark brown	dark brown gradually changing to dark red	yellow changing to brownish-orange then to pink
p-Xylenol	yellow quickly changing to orange, then orange-brown, then slowly to red	mahogany-brown then to brown-orange then soon to red	red solution with undissolved particles blue, on dissolving all red	orange-brown changing to green; fades slowly
Thymol	yellow changing to orange-brown then to dark brown	brown changing to deep red	red solution with undissolved particles blue; on dissolving all red	yellow changing to bright green
Carvacrol	orange-red changing to deep red	dark brown changing quickly to orange-brown then gradually to deep red	brown develops quickly, gradually changing to deep red	yellow-brown insoluble matter changing to light brown
Tyrosin (p-hydroxy-phenyl ethyl-amine)	red-brown develops quickly	strong dark brown	red-brown changing to dull red, then gradually to brown	yellow
Tyrosin (p-hydroxy-phenyl α-amino propionic acid)	light brown, changing to purple-red	strikes dark brown dissolves light brown, develops dull red, changing to violet-red	strikes dark red, dissolves dull red changing to deeper purple-red	light yellow
α-Naphthol[1]	undissolved solid becomes yellow-brown, dissolving to olive-green solution, changing quickly to yellow-brown	olive green changing to olive-brown, and then to brown gradually	green changing to blue-green	undissolved solid becomes orange-brown, dissolves to bright green solution soon fading to brownish-yellow

[1]In concentrated sulphuric acid the solid strikes an orange color and finally dissolves to make a yellow solution.

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
β-Naphthol[2]	yellow-orange, changing at once to olive green, and then to dark green	olive-brown changing to dull green, and then to gray-brown	greenish-gray, gradually changing to blue; weak reaction	dirty green
Monophenols With Halogen Group				
o-Chlorphenol	purplish-pink changing to deep purple-red at once, insoluble	brown changing to red	brown changing to orange with some brown at center; brown at center becomes dark green; then whole solution gradually changes to orange-red	light yellow
m-Chlorphenol	scarlet; mostly insoluble matter at first	brown changing to scarlet; mostly insoluble matter at first	brown changing to pink then to red	light yellow
p-Chlorophenol	strong green changing to dull dark green insoluble matter	strikes brown-purple dissolves brownish-yellow, soon changing to green	dull violet red; a white scum soon forms on the surface of the solution	light yellow
Tribromophenol	With concentrated sulphuric acid alone or the reagents a slight brownish coloration developed.			
Thymol iodide	Scarcely soluble at first in concentrated sulphuric acid or the reagents but gradually changing to light brownish.			
Monophenols With Nitro Groups				
o-Nitrophenol	With concentrated sulphuric acid alone or the reagents a yellow coloration developed.			
m-Nitrophenol	With concentrated sulphuric acid or with the reagents a yellow color developed.			
p-Nitrophenol	With concentrated sulphuric acid alone or the reagents slight yellowish color developed.			
Trinitro-phenol	negative	negative	negative	negative
1-Nitro 2-Naphthol	With concentrated sulphuric acid alone or the reagents a brownish yellow color developed.			
Monophenols With Amino Group				
p-Amidophenol[3]	dark blue	light gray-blue changing to green	purple with tinge of black changing to dull blackish green	dark blue
Amidol[4] (1, 3-diamino 4-hydroxy benzene dihydrochloride)	dark blue	purplish-blue	black tinge changing to dull black-purple	black-violet
Metol (monomethyl-p-amido m-cresol sulphate)	negative	yellow	light yellow	slightly yellow

[2]In concentrated sulphuric acid the solid strikes red orange, finally dissolving to make a yellow solution.

[3]In concentrated sulphuric acid a dark blue color is formed.

[4]In concentrated sulphuric acid a dark blue color is produced.

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Monophenols With Nitro and Amino Groups				
Pieramic acid (dinitro amino phenol)	With concentrated sulphuric acid alone or with the reagents, a light brown color is produced.			
Ethers of Monophenols				
Anisol (methyl phenyl ether)	pink changing to deep dark red	purplish-red	brown changing to olive-green, soon to red	brownish-orange tint changing to green then to pink
Anethol[5] (p-propenyl anisol)	yellow-brown	yellow-brown	brown, changing t dirty violaceous-brown then to black	orange-red
Phenetol (ethyl phenyl ether)	pink changing to deep bright red	violet red gradually changing to deep red	pink and brown changing to red and olive-brown, then to deep carmine-red	faint yellow changing to light green
Phenacetin (acetyl derivative of p-amino phenetol)	gradually very faint brownish-yellow	faint purple changing to purplish-green changing to faint brownish, reaction very weak	faint lavender, very, very weak reaction	faint yellow
Thyroxin (tetra-iodo-p-hydroxy phenyl ether of tyrosine)	brown	orange-brown	negative	negative
Nitrated Ethers of Monophenols				
m-Nitro-phenetol	With concentrated sulphuric acid alone or with the reagents, a yellow-orange coloration developed.			
p-Nitro-phenetol	With concentrated sulphuric acid alone or with the reagents a yellow color developed.			
Monophenols With Alcohol Group				
Diathesin[6] (o-hydroxy benzyl alcohol)	insoluble; red changing to violet	insoluble; purple	insoluble particles red-violet; solution becoming light pink	insoluble; particles dark red
Monophenols With Aldehyde Group				
p-Hydroxybenzaldehyde	negative	negative	negative	negative
Anisic aldehyde (p-methoxy-benzaldehyde)	With concentrated sulphuric acid alone or with the reagents a weak yellow-brown developed.			
Salicylic aldehyde (o-hydroxy benzaldehyde)	Concentrated sulphuric acid alone or the reagents produce a brown-yellow color, changing at once to deep orange-red.			

[5]In concentrated sulphuric acid alone an orange-red insoluble sticky mass is formed.
[6]In concentrated sulphuric acid alone insoluble particles turn red and solution turns pink.

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Monophenols With Carboxyl Group				
Salicylic acid (o-hydroxy-benzoic acid)	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink changing to orange	negative
Aspirin (acetyl salicylic acid)	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink, changing to orange	negative
m-Hydroxy-benzoic acid	light yellow	faint green	faint green	faint yellow
p-Hydroxy-benzoic acid	faint orange	faint salmon, orange develops	faint orange develops	negative
b-Oxynaphthoic acid[7]	soon becomes green	brown, changing to dark olive green, which soon changes to dark olive	brown changing rather slowly to olive	negative
Monophenolic Ethers With Carboxyl Group				
Anisic acid (p-methoxy-benzoic acid)	With concentrated sulphuric acid alone or with the reagents, a slight blue-green color developed.			
Esters and Salts of Phenolic Acids				
Methyl salicylate	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink changing to orange	negative
Salol (methyl salicylate)	red soon develops	brown-orange changing at once to red	develops salmon pink gradually changing to orange	light yellow, changing to light green
Sulphosalicylic acid	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink, changing to orange	negative
Sodium salicylate	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink, changing to orange	negative
Sodium or zinc phenolsulphonate	salmon-orange, changing to deep orange-red	brownish-orange, gradually changing to orange-red	gradually develops orange tint, changing to orange-red	negative
Diphenols				
Pyrocatechin (o-dihydroxy benzene)	dirty red changing to dirty purple	dark brown changing to brownish-violet, then to dark brown	dark brown changing to dark brownish-red with blue edge above level of solution	brown changing to olive then to olive green
Adrenalin[8] (o-Dihydroxy-phenol hydroxy ethyl-methyl-amine)	becomes brown	strikes dark brown dissolves orange-brown	orange-brown	yellow

[7]With concentrated sulphuric acid alone, yellow color formed.

[8]In sulphuric acid alone the solid particles strike a red color and finally form a light yellow solution.

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Resorcin (m-dihydroxy-benzene)	yellow changing to orange-yellow solution with scarlet flecks, gradually orange solution	brown changing to scarlet with deeper red flecks	brown changing to dirty purple then deep blue, then solution red at edge, blue at center	yellow; violet develops and changes to strong violet-blue
Orcinol (methyl resorcinol)	yellow changing to orange-brown	yellow-brown changing to orange-brown	brown changing to brown-red	yellow or brown-yellow
Hydroquinone (p-dihydroxy benzene)	brown	bright orange soon changing to brown-orange, then gradually to orange-red	brownish-purple changing to brown-red	brownish-yellow
Ethers of Diphenols				
Guiacol[9] (monomethyl ether of pyrocatechol)	deep red changing to dull red-violet	dark brown changing to dark purple	dark brown quickly changing to dark red	strikes green-brown, dissolves brown
Guiacol carbonate	pink changing to carmine	rose red changing to bright red	develops pink	negative
Eugenol[10] (allyl 3, 4, guiacol)	becomes dark red changing gradually to brown then to dull violet-red	dark brown changing to dark purple-red then dark brown	dark brown changing to dark brown-red	strikes brown-yellow, dissolves deep orange-red
Ethers of Diphenols With Alcohol Group				
Vanilly[11] alcohol (m-methoxy-p-hydroxy benzyl alcohol)	dark red changing to dirty dull violet	purple changing to dark blue then to black	dirty dull violet	red changing to dull red-violet
Ethers of Diphenols With Aldehyde Group				
Vanillin m-methyoxy-p-hydroxy-benzaldehyde	With concentrated sulpheric acid alone or with reagents a light yellow color developed.			
Piperonal (heliotropin-anhydride of vanillin)	With concentrated sulphuric acid alone or with reagents a yellow color developed.			
Veratraldehyde	With concentrated sulphuric acid alone or with reagents a yellow color developed.			
Ethers of Diphenols With Carboxyl Group				
Vanillic acid (m-methoxy p-hydroxy benzoic acid)	yellow orange changing to orange-red	brown-yellow changing to green with yellow border	yellow-brown develops gradually	yellow

[9]In concentrated sulphuric acid alone a brown color appears.
[10]In concentrated sulphuric acid alone a deep orange red is formed.
[11]In concentrated sulphuric acid alone a deep carmine red is formed.

TABLE II—CONT'D

Triphenols

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Pyrogallol (1, 2, 3, trihydroxy, benzene)	bright orange-red changing to dark red-brown	dark brown changing brownish-red	dark brown changing to reddish-brown	reddish, changing at once to brown, then gradually to dull green
Phloroglucin (1, 3, 5, trihydroxy benzene)	bright orange changing to scarlet	yellow changing to orange-red	particles red, gradually dissolves to deep purplish-red	slightly yellow

Triphenols With Carboxyl Group

Gallic acid (3, 4, 5, trihydroxy benzene)	light brown-orange changing to green	brown changing to dull green, gradually changing to dark red	olive green changing to brown	yellow-brownish
Tannic acid[12] (digallic acid)	orange-brown changing to dark brown, insoluble particles poor reaction	dark brown, insoluble particles, poor reaction	dark brown, insoluble particles, poor reaction	pink-brown changing to brown; poor reaction

Salts of Triphenolic Acids

Dermatol (bismuth subgallate)	light yellow	orange-red changing gradually to brown-orange	orange changing gradually to red	light yellow-brown changing to light brown

Glucosides Yielding Phenols on Hydrolysis

Arbutin (yields hydroquinone on hydrolysis)	brown	brown changing to brownish-orange	deep purple changing to brown quickly	yellow changing to brownish-yellow
Salicin[13] (yields on hydrolysis saligenin or o-hydroxybenzyl alcohol)	strong red changing to dark dull red	deep red	bright scarlet	orange-red
Phloridzin[14] (yields on hydrolysis the phloroglucin ester of p-hydroxyatropic acid)	yellow changing to brown	brown	red-brown changing to orange-brown	light yellow
Aesculin (yields on hydrolysis aesculetine or 4, 5 dihydroxy coumarin)	With concentrated sulphuric acid alone or with the reagents a faint yellow color develops.			

Miscellaneous Compounds

Brucine	negative	strikes red, dissolves without color, orange develops, changing to yellow	strikes red, dissolves orange, changing to yellow	strikes red, dissolves orange, changing to yellow

[12]In concentrated sulphuric acid a brownish yellow color develops.

[13]In concentrated sulphuric acid alone a red color is produced.

[14]In concentrated sulphuric acid alone a light yellow color develops which changes to light orange.

TABLE II—CONT'D

MISCELLANEOUS COMPOUNDS—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Diphenyl-amine[15]	negative	intense deep blue	intense deep blue	deep blue
α-Naphthal-amine	green or blue-green develops	brownish changing to dirty dark green, then to dark brown, then to dark brown-red with violet tint	solution gradually becomes light brown with lavender edge, changing to purple-red, then slowly to deep red	faint yellow, fading
β-Naphthyl-amine	deep blue develops, quickly changing to blue-green	beautiful blue soon develops, changing to deep blue-green gradually	yellow-brown develops gradually	faint yellow
Benzidine	With concentrated sulphuric acid alone or with reagents faint purple changing to slight red developed.			
Phenyl-hydrazine hydrochloride	orange soon develops	orange-yellow develops, weak reaction	becomes slightly greenish	yellow
Tryptophane[16] (β-indol α-amino propionic acid)	brown	light brown	yellow brown, changing to orange	yellow
Ephedrine	negative	negative	negative	negative

[15]In concentrated sulphuric acid alone a light dull green develops.
[16]The reactions recorded are very weak.

and their derivatives, triphenols, and phenolic glucosides. A brief discussion of the results obtained follows.

The four reagents react with phenols, ethers of phenols, and other phenolic compounds.

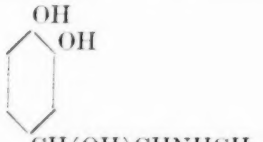
The Marquis reagent is useful for phenols in general. Most of them give red or brown colors.

Erdmann's reagent gives comparatively weak reactions. It may, however, be used to very great advantage with resorcin, and is also of some use with thymol and various other phenols.

Fulton's Reagent A gives distinctive and striking reactions with the opium alkaloids, quite different from those given by Marquis' reagent. With most phenols, however, it gives reactions similar to those with the Marquis reagent, and where this is the case it has no advantage over the latter. Reagent A is easily prepared.

Fulton's Reagent B requires a little practice to prepare properly, and has to be tested on known morphine and codeine (or on a known sample of the substance for which the test is made) before it can be used. In many cases, however, this reagent will be well worth the trouble of preparation. It is useful for the opium alkaloids, especially in testing for codeine or dionine. With the majority of phenols the reactions are very similar to those with the Marquis reagent or Reagent A. Distinctive and characteristic reactions, however, are obtained with phenol, p-xylenol, α-naphthol, β-naphthol, resorcin, and pyrocatechin.

Adrenalin is a diphenol. Ephedrin behaves physiologically like adrenalin, but it has no phenolic groups within its molecule. The formulas for the two compounds are as follows:

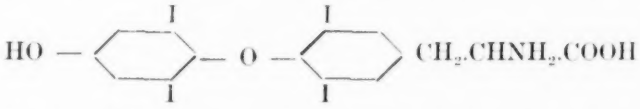


Adrenalin

Ephedrin

The similarity in the composition of the side chains in these two compounds indicates the common factor responsible for their resemblance in physiologic behavior. From our standpoint it is interesting to note that the nonphenolic compound, ephedrin gives negative reactions with our four reagents, while adrenalin, a diphenol, gives positive reactions. The four reagents can, therefore, be employed for the chemical differentiation of adrenalin and ephedrin.

Thyroxin, according to E. C. Kendall,[4, 5] was believed to be a compound derived from tryptophan. Harington,[6, 7] however, has recently proved that the thyroid hormone is derived from the phenolic amino-acid, tyrosin. According to his researches, thyroxin is a tetra-iodo derivative of the p-hydroxyphenyl ether of tyrosin. The formula of this phenolic ether is given below:



The positions of the iodine atoms in this ether molecule have been determined by Harington Barger.[8] The iodine atoms occupy the 3,5,3′,5′, positions. It is interesting to note that the positive reactions we obtained with thyroxin with the Marquis reagent and with Fulton's Reagent A harmonize with Harington's findings in reference to the phenolic character of this hormone.

The two naphthylamines give good reactions with Reagents A and B. Since the color responses are different, either or both of the reagents can be used to differentiate the alpha compound from the beta compound.

The presence of the aldehyde group in the phenol compounds is the cause of negative reactions with all the four reagents. This has been the experience with para-hydroxybenzaldehyde, para-methoxy-benzaldehyde (anisic aldehyde), o-hydroxy-benzaldehyde (salicylic aldehyde), with vanillin, with piperonal, and with veratraldehyde.

The presence of the nitro group is also inimical to the formation of a color reaction with any of the four reagents. Negative results have been obtained with o-nitrophenol, m-nitrophenol, p-nitrophenol, trinitrophenol, dinitro-amino-phenol, m-nitro-phenetol, and p-nitro-phenetol.

A comparatively large number of halogen atoms in the molecule render the reagents inert. Tribromophenol and di-iodothymol (thymol iodide) do not respond to the four reagents. On the other hand, o-chlorophenol, p-chlorophenol, and m-chlorophenol have been found to be reactive.

TYPES OF REACTIONS

A great variety of effects are obtained with the four reagents.

With morphine and with codeine each of the reagents give a different reaction, although the reaction of Reagent B somewhat suggests a very sensitive Erdmann reagent. Reagent A gives a reaction entirely different from that of either the Marquis reagent or Reagent B.

With oxydimorphine the reactions of Reagents A and B are similar, and bear a general resemblance to the Erdmann reaction, but all are entirely different from the reaction with Marquis reagent. In this case it will be found that adding any oxidizing agent along with the Marquis reagent, such as a small crystal of potassium permanganate or chromate, will change the reaction entirely and make it similar to that of Reagent A. This is not the case with morphine or codeine.

With m-cresol, and with many other phenols, including m-xylenol (2), carvacrol, veratrol, and phenetol, the reactions of the three reagents containing formaldehyde are very similar, while Erdmann's reagent also reacts. The colors with the formaldehyde reagents are usually red or brown.

With resorcin, and with a number of phenols, including p-xylenol, thymol, phenol and anisol, the reaction of Reagent A is very similar to that of Marquis' reagent, and usually less distinctive, while the reaction of Reagent B is decidedly different, and the most distinctive of the four.

Salicylic acid and similar compounds do not react with Erdmann's reagent, and the reactions with Reagents A and B are weaker than with the Marquis' reagent. Brucine and diphenylamine do not react with Marquis' reagent, but with Reagents A and B give reactions similar to those with Erdmann's reagent.

POSSIBILITY OF MISTAKING A PHENOL FOR AN OPIUM ALKALOID

With Marquis' reagent most of the opium alkaloids give purple colors, while most of the phenols give red or brown. However, some phenols do give purple colors.

A number of phenols have been found which give with Marquis' reagent reactions more or less like those of morphine. These include pyrocatechin, guaiacol, vanillyl alcohol, and veratrol. These substances, however, all give precipitates on diluting the reaction-mixture with water, while morphine and codeine give clear yellow solutions. We have not confirmed the previous observation that p-cresol also reacts like morphine.

Reagent A gives a very valuable specific reaction with morphine. The reactions of phenols in general are quite different. It is probable that no compound exists which gives the same reaction as morphine with this reagent, save, of course, such closely related derivatives of morphine itself as heroine. Only one compound, β-naphthol, has been found as yet which gives with Reagent A a reaction at all similar to that of codeine.

With Reagent B codeine gives a distinctive and characteristic reaction. Again β-naphthol shows a certain resemblance, while di-phenylamine might

easily be mistaken for codeine so far as this one reaction is concerned. Reagent B is not of much value for morphine.

The three reagents containing formaldehyde identify not only morphine and heroine, codeine and dionine, but also oxydimorphine, apomorphine, papaverine, and narcotine. By considering the reactions of these opium alkaloids with the three reagents it should be possible to identify each of them beyond a reasonable doubt.

SUMMARY

The Marquis reagent, the Erdmann reagent, and the Fulton reagents react with phenols, ethers of phenols, phenolic alkaloids, and other phenolic compounds. They can be applied in a practical way to identify these compounds.

These reagents are not absolutely specific for phenols, since certain nonphenolic compounds, α and β-naphthylamine, phenylhydrazine, and tryptophan, also yield reactions.

The presence of the aldehyde group in the phenol compound inhibits the color reactions obtained with the four reagents.

The presence of the nitro group is also inimical to the formation of a color reaction.

Tribromophenol and di-iodothymol do not respond to the four reagents, but the monochlorophenols have been found to be reactive.

The four reagents can be utilized to differentiate the opium alkaloids; to distinguish the phenolic hormone adrenalin from the nonphenolic compound ephedrin; to distinguish tyrosin from the nonphenolic amino acids; and to differentiate alpha naphthylamine from the beta form.

The three reagents containing formaldehyde are the most valuable. Erdmann's reagent gives comparatively weak reactions.

Some of the phenols give some reactions similar to those of morphine, codeine or other opium alkaloids. However, by using the three formaldehyde reagents, or all four reagents, it should be possible to identify morphine, codeine, oxydimorphine, apomorphine, papaverine, and narcotine beyond a reasonable doubt.

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LABORATORY METHODS

NEW METHOD FOR RECORDING MINUTE FLUCTUATIONS OF FLUIDS IN CAPILLARY TUBES WITHOUT A FLOATER*

By Maurice Muschat, M.D., Philadelphia, Pa.

RECORDING fluctuations of liquids becomes quite a problem when the manometer is a capillary tube and the excursions of the moving fluid level are small. A floater cannot be used in such cases.

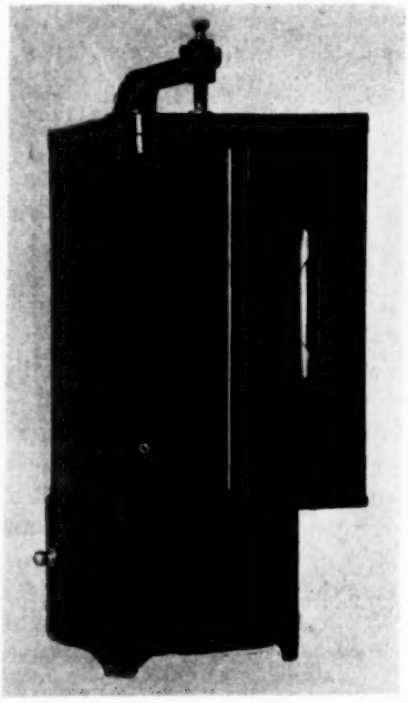

Fig. 1.—Complete outfit of the recording camera.

While working on the contractions of the milking muscle of the kidney, the necessity arose for obtaining a permanent record of the up and down movements of the fluid in a capillary, and we therefore devised the following apparatus.

An ordinary revolving drum was used. A metal box with a removable lid was constructed, measuring in width and height 2 cm. more than the re-

*From the Department of Research Surgery, University of Pennsylvania.
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volving drum. Two openings were made; one in the center of the bottom, the other in the middle of the lid, in order to permit the central axial rod of the drum to carry the cylinder inside of the metal box. On the frontal side of the box a slit, extending all the way down was made with a lateral sliding bar to make the slit as wide as desired. A metal strip slides over the slit and completely darkens the inside of the box. (Fig. 1.)

The box with the revolving drum inside of it fits into the main drum-stand by means of the lower end of the axial rod which falls into the slot, and the upper end which fits into the arm of the stand and is fastened by means of a screw. (Fig. 2.)

The box containing the revolving drum, is easily removed from the stand and carried into a dark room, where it is loaded. A piece of bromsilver paper

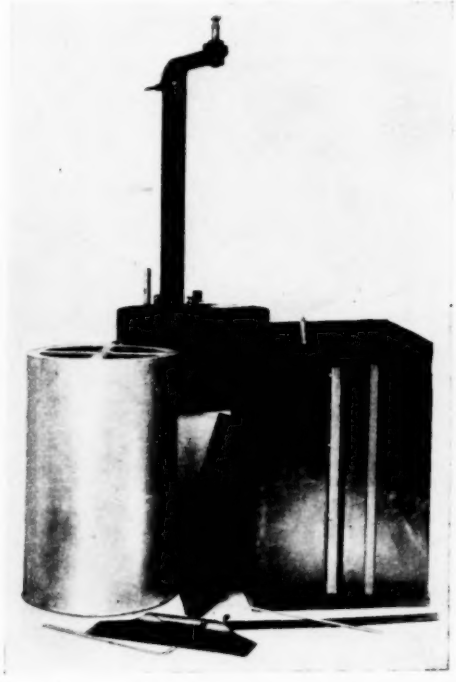

Fig. 2.—Recording camera taken apart, to show the various parts.

having been cut to the size of the drum, is now put around the removed drum and held in place by two rubber bands. The drum is then replaced in the box by sliding it over the axial rod. After having reached the bottom of the box, the drum is brought up for about 2 cm. and the upper holding screw tightened. The lid is then fit over the top and the outer screws tightened. The box can now be taken into the light and put back on the stand ready for use. Having thus constructed the receiving camera, the problem was to have something on top of the moving fluid in the capillary in order to get a white or black line on the recording paper.

Capillaries with a diameter of 0.5 mm. are entirely too small to carry any kind of a floater, even a small piece of dark glass sticks to the walls of

the tube. A drop of a yellow liquid lighter than water and insoluble in water floating on top of the moving liquid of the capillary would answer the question. At a suggestion by Dr. E. Wagner of the Department of Chemistry these requirements were met by using a solution of azo-benzene, a dark yellow organic substance, dissolved in benzene. This solution does not mix with water, and being reddish-yellow in color excludes the penetration of any light through the floating drop.

It is also worth noting that the azo-benzene itself is entirely insoluble in water, which gives the assurance of the yellow drop not mixing with the important oxygenated physiologic solutions used in the experiment and in this way eliminates the possible interference with the actual preparation. The solu-

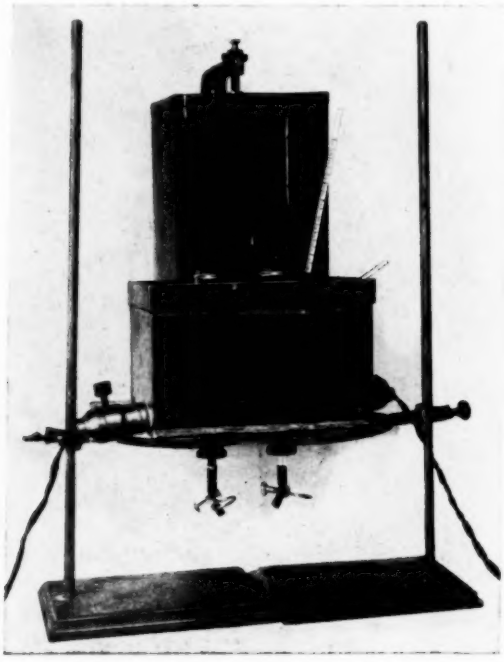

Fig. 3.—Recording camera in action in conjunction with a constant temperature bath.

tion of azo-benzene in benzene is aspirated into very fine capillaries which fit into the manometer tube. Having sealed one end, these loaded capillaries are kept ready for use.

After having prepared the main part of the experiment and being ready to record the obtained fluctuations, one of the small capillaries with the yellow liquid is inserted into the upper end of the manometer tube and brought down until it reaches the fluid level, the upper end of the capillary is then broken off and the yellow liquid is permitted to run out on top of the manometer fluid. In order to obtain a good record, it is best to put in a yellow column of 1 cm. in height.

The capillary manometer is now brought close to the recording box and pushed into the frontal slit. (Fig. 3.) Black paper strips are being used to

cover the upper and lower parts of the slit, where the penetration of light is not desirable. A piece of metal strip with a 0.5 mm. slit slides in front of the manometer to reduce the light, and to obtain a clear picture. A 60 watt frozen bulb is used facing the box at a distance of 30 cm. The opening of the

Fig. 4.—Chart showing recorded fluctuations, that were hardly noticeable with naked eye.

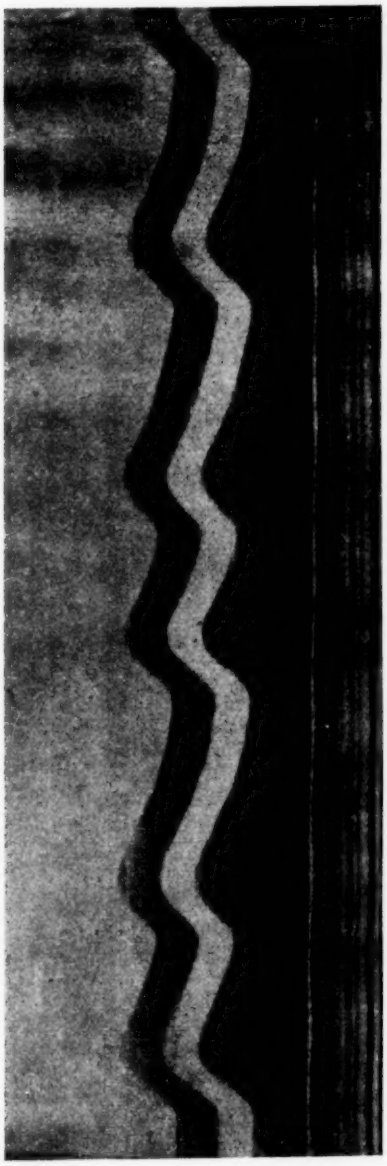

Fig. 5.—Chart showing heavy fluctuations recorded by the camera.

lamp shade is covered with a piece of pasteboard with a central opening of about 3 cm. in diameter.

After the capillary manometeric tube has been set into the slit of the box, the proper screening accomplished, the light turned on and focused, the drum

is made to run as slowly as desired, recording every movement of the yellow drop in the capillary. It is obvious that movements in several tubes could easily be recorded in this way simultaneously.

Two records are appended (Figs. 4 and 5), one where the fluctuations were hardly noticeable; the second where stronger movements were seen. The time can easily be recorded either by turning out the light or by placing something in front of the slit every minute.

AN APPARATUS FOR AUTOMATICALLY ELICITING AND RECORDING THE ACHILLES JERK*

By W. W. Tuttle, Ph.D., Iowa City, Iowa

THE susceptibility of the deep reflexes to changes in the neuromuscular mechanism has brought forth a vast amount of research dealing with the factors responsible for these changes. Up to the present time most of the quantitative investigations have been based upon the knee jerk of both animal and human subjects. It has been demonstrated in this laboratory that the Achilles jerk is just as reliable as an index to neuromuscular changes as is the knee jerk. Furthermore there are a number of advantages in favor of using the Achilles jerk.

For the quantitative study of the Achilles jerk an apparatus has been devised using principles identical to those involved in the apparatus developed for the eliciting and recording the knee jerk (Tuttle, 1924).

The apparatus consists of the following three parts: (1) the stimulating unit; (2) the subject unit; (3) the recording unit.

The Stimulating Unit.—The details of this unit are shown in Figs. 1 and 2. Essentially it consists of a motor, gear-box, tripping device and stimulating hammer mounted on an adjustable stand. The speed of a 1750 R.P.M. motor is reduced to 10 R.P.M. by means of a worm gear. This reduction necessitates a ratio of 1:175 between the number of teeth in the worm and the number in the gear. To reduce the noise the worm gear runs in grease. A wooden wheel 8 inches in diameter is attached to the shaft of the gear. The stimulating hammer is mounted in bearings so that the hammer handle extends past the pin placed near the edge of the wooden wheel. As the wheel turns the pin engages the hammer handle, lifts the hammer, dropping it when the pin ceases to engage the handle. A stop supports the hammer when the machine is not in motion.

The apparatus is flexible with regard to both rate and strength of stimuli. The hammer handle is provided with a weight which serves to vary the strength of the stimuli as its position with relation to the fulcrum is changed. An increase in the rate of stimulation is brought about by adding a pin to the tripping wheel. If an additional pin is placed exactly opposite the one already

*From the Department of Physiology, State University of Iowa, Iowa City.
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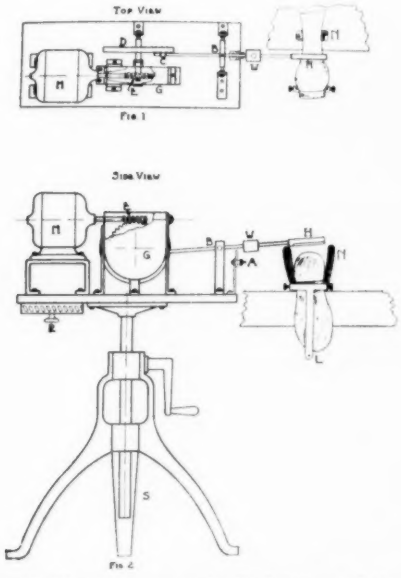
in the wheel the rate of stimulation is uniformly doubled. A rheostat in the motor circuit serves to control the speed of the motor. By adjusting this rheostat one may secure any desired speed slower than 10 R.P.M.

The Subject Unit.—Although detailed drawings of this unit are not included, the arrangement of the leg and foot for the delivery of the stimuli to the Achilles tendon is shown in Figs. 1 and 2. The subject is placed in a prone position on a well-padded table. A support is fastened to the table to hold the leg and foot secure. In order that the member involved may be free to move, the observer is placed so that his foot extends well over the edge of the table. This position is also advantageous since it is at once suitable for the application of the stimuli and the recording of the response. The stimulating



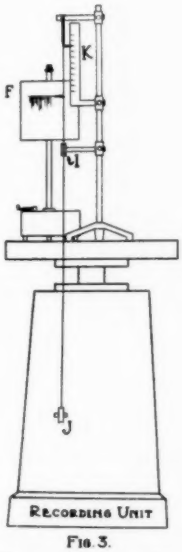


Fig. 3.

Fig. 1.—*A*, Hammer support; *B*, fulcrum of the hammer; *E*, worm-gear; *G*, gear box; *H*, hammer; *L*, T-clamp.

Fig. 2.—*M*, Motor; *N*, leg support; *R*, Rheostat; *S*, adjustable stand; *W*, weight.

Fig. 3.—*F*, Kymograph; *I*, guide pulley; *J*, guide pulley; *K*, iron stand.

unit is placed in proper proximity to the Achilles tendon and adjusted to the position suitable for the experiment.

The Recording Unit.—This unit is set at the subject's head. Its details are shown in Fig. 3. The record is made by attaching the toe of the shoe to a recording stylus as shown in Figs. 1 and 2. A T-clamp is fastened to the sole of the shoe close to the heel. Stout fish-line runs from a hole in the T-clamp under the table through guide pulleys to a rubber band suspended from an iron stand.

The importance of uniform tension on the toe is easily demonstrated by varying this factor. It is evident that within limits an increase in tension increases the extent of the reflex. In order that such a determinant may be controlled a pointer is attached to the line and a millimeter scale is fastened to

the iron stand. By adopting a uniform position for the pointer on the scale one controls the factor of tension.

The record is made on a slowly moving kymograph equipped with a clock release. When the jerk is elicited, the recording stylus is drawn down making a record comparable to the one shown in Fig. 3. When the toe returns to its original position, the elasticity of the rubber band pulls the stylus back to the base-line. Since the base-line is at the top, the records are read from right to left.

The displacement of the toe represented by vertical lines on the record furnishes an index to the extent of the reflexes. A mathematical index is established by finding the average in millimeters of a given number of responses. For all practical purposes ten stimuli per minute are delivered over a period of ten minutes. This gives a fair sampling of the responses of a subject.

SUMMARY

An apparatus has been devised for automatically eliciting and recording the Achilles jerk. It delivers uniform stimuli at a constant rate to the Achilles tendon. The displacement of the toe of the foot recorded as vertical lines on a slowly moving kymograph furnishes the index to the extent of the reflex. A mathematical index is established by finding the average height in millimeters of a given number of responses.

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A NOTE ON THE STANDARDIZATION OF ANTIGEN FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By A. Schaible, A.B., Albany, N. Y.

DURING the past year or more, in standardizing antigens for use in the complement-fixation test for syphilis, considerable difficulty has been experienced in preparing successive cholesterinized extracts of uniform sensitivity by a method similar to that of Neymann and Gager.[1] Beef-heart tissue, which was used as the basis for these antigens, appears to vary in the lipoid content with the result that two antigens prepared by the same method from different lots of beef hearts frequently varied in sensitivity. Several successive antigens prepared by the same method were all less sensitive than the standard antigen. As it was thought that possibly the ether extraction had been somewhat excessive, in one or two subsequent antigens, a less thorough ether extraction was employed without, however, improving the quality of the resulting antigen. An attempt was made to bring one of these less sensitive antigens up to standard

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

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by increasing the percentage of cholesterin with which it was reinforced, and it was found that quite marked increase in the cholesterin content had little effect in increasing its sensitivity.

In view of the possibility that the lack of sensitivity of these antigens was due to overconcentration rather than underconcentration of lipoids, one of the less sensitive antigens was diluted with an equal part of absolute alcohol and then reinforced with the 0.4 per cent cholesterin routinely employed. The ordinary dilutions in salt solution were made, and the antigen was compared with the standard in a large series of tests, with gratifying results. Of five hundred and sixty comparative tests, there was approximate agreement in the results obtained with the two antigens in 96.6 per cent.

When several of the other less sensitive antigens were treated in the same way, the sensitivity of one was increased to that of the standard antigen by the addition of an equal part of alcohol; another required one and one-half parts of alcohol to bring it up to the standard and when two parts were added, it became more sensitive. Yet, when the standard antigen was diluted with an equal part of alcohol, its sensitivity remained unchanged. Thus, it appears that in some antigens the optimum concentration of lipoids covers quite a wide range. In all, five antigens diluted with alcohol have now been compared with the standard in a large series of tests and their sensitivity has been successfully adjusted by the addition of from one to one and one-half parts of alcohol.

The increase in sensitivity of the alcohol-diluted antigens appeared to be due, to some extent, to an increased instability of the final dilution in salt solution, as the dilutions of these antigens tended to become anticomplementary after they had stood for several hours. The increase in anticomplementary effect was more marked with some pools of guinea pig complement than with others. The dilutions were always satisfactory for as long as two hours and in most instances considerably longer. It is wise, therefore, to prepare a fresh dilution if any tests are to be made after the antigen dilution has been standing for a period of more than two hours. It is also advisable to give all antigen dilutions a period of approximately fifteen minutes after dilution with salt solution, to reach a state of colloidal equilibrium.

This method of increasing the sensitivity of an antigen by dilution with alcohol, which has been described in connection with the precipitation test,[2] seems of sufficient importance to justify publication of its application in standardizing antigen for use in the complement-fixation test.

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A COMPARISON OF THE RESULTS OF MEINICKE, KAHN, AND WASSERMANN REACTIONS*

By Hester A. Austin, B.S., and Margaret M. Frey, Rochester, N. Y.

AMONG the numerous precipitation tests used for the serodiagnosis of syphilis, the Meinicke turbidity reaction has been well recommended on account of its reliability and simplicity. This test has had a widespread use in Europe and is apparently gaining favor in tropical countries. In the literature of this country, however, there are only a few reports of the results of comparative tests with this procedure and the complement fixation and flocculation tests used to obtain serologic evidence of syphilis. This paper adds to the information on that point by presenting the results of a series of parallel tests with the Wassermann, Kahn, and Meinicke reactions on 1000 specimens of blood serum.

In the course of its development, the Meinicke test has undergone a number of modifications. Of these, the one which is now coming into more general use is known as the TR or turbidity reaction. It is described by Meinicke[1] in a paper published in 1927 in the Journal of Laboratory and Clinical Medicine. This article deals with the development of the test, and gives a bibliography of papers reporting experiences with it in attempts to evaluate its simplicity, specificity, and sensitiveness.

Among the recent articles not cited by Meinicke, we have found only three which report a comparison of the Wassermann, Meinicke, and Kahn tests.

Ruediger[2] reported examining 311 consecutive specimens with his modification of the Wassermann test, the Kahn test, and the Meinicke test with about 87 per cent agreement between the three tests. In a second paper, Ruediger[3] reported the examination of 265 consecutive specimens with a Kolmer-Wassermann test, Ruediger-Wassermann test, the Kahn test, and Meinicke test. There was 84.5 per cent agreement between the four tests; 91.6 per cent between the Kolmer-Wassermann, the Kahn and Meinicke tests; 86.8 per cent between the Ruediger-Wassermann, the Kahn and Meinicke tests. This lower percentage was due to the greater sensitivity of the Ruediger-Wassermann test. Schmidt and Ziekmann[4] reported the results of 500 consecutive specimens examined by the three tests with 96.2 per cent agreement. The Wassermann and Kahn reactions agreed in 97 per cent of cases, the Wassermann and Meinicke in 97.6 per cent, the Kahn and Meinicke in 97.8 per cent. They concluded that "the Kahn test in this laboratory has not been so sensitive as either the Wassermann or Meinicke turbidity reactions, and in one case a false positivity was obtained. The clinical evidence in this study indicated a higher degree of specificity for the Meinicke turbidity reaction than for the Kahn test."

There are a number of reports comparing the results of the Wassermann and Meinicke tests. Ledermann[5] in an examination of 504 serums found 93

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per cent agreement. Rubenstein and Suarez[6] reported 90 per cent agreement in 490 serums; they concluded that the Meinicke test was specific in cases with strong reactions but a little less sensitive than the Wassermann, and that doubtful reactions were not always specific. Saunders[7] reported 94.5 per cent agreement in 1000 blood serums examined. Brugh, Landon and Laird[8] examined 500 specimens finding about 95 per cent agreement between the tests. Blumberg[9] reported 98 per cent agreement in 1500 specimens. Mefford[10] reported that in an experience of over 3000 tests, he found the Meinicke reaction more reliable because it was more sensitive than the Wassermann test. A positive reaction occurred earlier and persisted longer in treated cases. The Meinicke test gave positive reactions in late or recurrent syphilis when the Wassermann test was negative. Gross[11] reported 91.6 per cent agreement in 1500 serums. Rucker and Whitby[12] reported 91 per cent agreement in 254 serums using an antigen prepared by themselves; and 90 per cent agreement in 160 serums using Meinicke's own reagent. They considered the Meinicke test easier to perform and to read than the Kahn test. In their opinion, the value of the Meinicke test was "mainly for orientation, the progress of treatment being controlled by the Wassermann test with or without Meinicke's reactions as confirmatory evidence." Borowskja[13] found 98 per cent agreement between the two tests in an examination of 548 serums.

Our series consisted of 1000 serums, the majority of which were chosen because they gave positive reactions with either or both the Wassermann or Kahn tests, which are carried out routinely in our laboratory. The serums were clear, neither distinctly hemolyzed nor chylous; they were inactivated for one-half hour at 55 to 56° C., after being withdrawn from the clots. If used twenty-four hours, or more, later, they were reheated at 55 to 56° C. for ten minutes. Meinicke[1] advised using unheated serum, while Mefford[15] found that inactivation of the serum made little difference in the results. The Wassermann test was regarded as our standard and Meinicke or Kahn tests not agreeing with it, were repeated.

The technic used in the Wassermann test was similar to that used in the New York State Laboratory. The total volume of the test was 0.5 c.c., i.e. one-tenth that of the original Wassermann test. All reagents were diluted in accordance with preliminary titrations, so that a quantity of 0.1 c.c. might be pipetted in the test. The complement was titrated daily with 5 per cent sheep cells sensitized with two units of amboceptor; and two units of complement were used in the test. Each specimen was tested with two antigens, an alcoholic extract of beef heart, reinforced with 0.4 per cent cholesterin; in addition to this antigen, for about 200 of the 1000 tests, a crude alcoholic extract of beef heart was used; for the rest we have used an acetone-insoluble extract of beef heart prepared according to Bordet's method. Antigens and amboceptor were supplied by the State Laboratory at Albany, New York. The tests with both antigens were kept for four hours at 3 to 6° C. for the period of fixation. Five per cent sheep cells, sensitized with two units of amboceptor were then added, and the tests placed in the water-bath at 37° C., for fifteen minutes (until controls with serum alone, and antigen alone, showed complete hemolysis). Readings were then made, using a color standard, all tubes showing

2+ or more being centrifugalized, and the percentage of inhibition of hemolysis read from the supernatant fluid. About 150 tests were done each day.

The Kahn tests were performed with antigen prepared in our laboratory, Dr. Kahn very kindly checking our titration. The method used differed from the regular Kahn technic[14] only in the use of a two-tube instead of a three-tube test, omitting the tube containing 0.05 c.c. of antigen. This was done in order to secure uniformity. Many specimens did not provide sufficient serum for three tubes. In addition, from 5 to 10 per cent of the Kahn tests were one-tube tests, using 0.01 c.c. antigen mixture and 0.1 c.c. of serum. The result with these proportions of serum and antigen approximated the results of the two-tube test. All tests were read by two workers.

The Meinicke technic was that outlined by Mefford;[13] and the antigen was obtained from him. In view of the statements of Meinicke and others[12] that the efficacy of the antigen is dependent upon the properties of certain preparations of balsam of Tolu, we did not attempt to prepare the antigen in this laboratory. One c.c. of antigen was placed in one 15 c.c. centrifuge tube, and 10 c.c. of 3 per cent salt solution in another; the two tubes were put in a water-bath at 40° C. to 45° C. for not less than ten nor more than twelve minutes. During this period, 0.2 c.c. of each serum to be tested was pipetted into a tube. The formalin control was omitted. At the specified time, the antigen and saline were mixed by pouring the saline into the antigen, and back and forth twice. One c.c. of antigen mixture was added to each tube of patient's serum, while still warm. The tubes were placed in the dark at room temperature for one hour and then read. The positive reactions showed varying degrees of opacity. We did not run controls in this series of tests, but examined the serums themselves for cloudiness, in case of doubtful readings. We read the tests, sitting at about four feet from the window, holding the rack of tubes against the light. To one accustomed to reading Kahn tests, the Meinicke readings were comparatively easy. All tests were read by two workers. The tests were then left overnight at room temperature, and were read again. After this period negatives appeared as unchanged, while strong positives (4+) had a clear fluid and heavy sediment, and 3+ and 2+, were progressively less clear.

PRESENTATION OF RESULTS

For our tables, 2+, 3+, and 4+ readings have been grouped as positive, and ±, and + as doubtful.

Table I shows the results of the three tests on 1000 specimens examined. There appears to be very little difference in the results obtaind by the three tests. The Kahn test shows a slightly smaller number of doubtful reactions, as read by us.

TABLE I

RESULTS OF TESTS ON ALL SPECIMENS

	WASSERMANN	MEINICKE	KAHN
Positive	564	560	566
Doubtful	52	59	41
Negative	384	381	393
	1000	1000	1000

Table II shows the percentages of agreement between the three tests, and between each pair of tests. The percentage of agreement between the three tests is 81.5 per cent, which is lower than that apparently indicated by Table I. This is due to the fact that several specimens gave positive reactions with two of the tests while failing to react with the third. Agreement between the Wassermann and Kahn tests and between the Meinicke and Kahn tests appears to be about the same, while that between the Wassermann and Meinicke tests is somewhat lower.

TABLE II

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF TESTS

Agreement	WR. AND K. AND M.		WR. AND K.		WR. AND M.		K. AND M.	
Positive or doubtful	510		536		546		553	
Negative	305		336		312		328	
Total Agreements	815	81.5%	892	89.2%	858	85.8%	881	88.1%
Disagreement								
WR. pos. or doubtful K. neg.			59					
WR. " " " M. "					69			
K. " " " WR. "			49					
K. " " " M. "							66	
M. " " " WR. "					73			
M. " " " K. "							53	
Total Disagreements	185	18.5%	108	10.8%	142	14.2%	119	11.9%
	1000	100.0%	1000	100.0%	1000	100.0%	1000	100.0%

TABLE III

CORRELATION OF RESULTS WITH HISTORIES

	WR.		M.		K.	
Routine Cases						
Positive or doubtful	192	38.3%	206	41.1%	193	38.5%
Negative	309	61.7%	295	58.9%	308	61.5%
	501	100.0%	501	100.0%	501	100.0%
Untreated Cases and Cases With Suggestive Histories						
Positive or doubtful	74	69.8%	78	73.6%	76	71.7%
Negative	32	30.2%	28	26.4%	30	28.3%
	106	100.0%	106	100.0%	106	100.0%
Treated Cases						
Positive or doubtful	350	89.1%	335	85.2%	338	86.1%
Negative	43	10.9%	58	14.8%	55	13.9%
	393	100.0%	393	100.0%	393	100.0%

We divided the thousand specimens according to histories into (1) routines, (2) untreated cases and those with suggestive histories, and (3) treated cases. Routines included those with no history, or a history not suggestive of syphilis. We purposely selected the greater number of our specimens from among those giving positive readings with the Wassermann or Kahn test, as stated previously. Table III shows the results of the three tests on the specimens so grouped. In routine cases there appears to be almost complete agreement between the Wassermann and Kahn tests, which show a smaller number of positives, than

does the Meinicke test; in untreated cases or those with suggestive histories, the results with the three tests agree very closely, while in treated cases, the Wassermann test is more sensitive than the Meinicke and Kahn tests, which agree closely. Briefly, the Wassermann test demonstrates its equality with the other two tests, with the three classes of specimens, and a slight superiority to both, in treated cases.

SUMMARY

Our percentage of agreement between the three tests, 81.5 per cent was lower than the figures presented by Ruediger, 87 per cent and 86.8 per cent, and by Schmidt and Zickmann, 96.2 per cent. This may be largely accounted for by the fact that our series contained a much smaller number of specimens which were negative by all three tests.

Our percentage of agreement between the Wassermann and Meinicke tests, 85.8 per cent, was also lower than that reported by others. The reason given above may account for the discrepancy. The cases which disagreed were about equally divided between the two tests

The Meinicke test showed a few more positive reactions with routine specimens than either the Wassermann or Kahn tests. We have not yet determined, from clinical or other sources, whether or not these were "false positive" results.

In cases with histories suggestive of syphilis and untreated cases of syphilis, the results of the three tests agreed closely.

In treated cases of syphilis, the Meinicke test was as sensitive as the Kahn test while both were somewhat less sensitive than the Wassermann test.

CONCLUSIONS

The results of the Meinicke's turbidity reaction in the serodiagnosis of syphilis compare favorably with those obtained with the Wassermann technic recommended by the New York State Laboratory. The test is less sensitive than the complement-fixation reaction. In our opinion, it should not be used to supplant the Wassermann reaction at present. Its chief advantages, as compared with a complement-fixation test, are the facility with which it may be set up, and the simplicity and inexpensiveness of the reagents and apparatus required.

The results obtained with Meinicke and Kahn tests are closely parallel.

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A RAPID STAIN FOR THE DIRECT MICROSCOPIC EXAMINATION OF MILK*

By Norris M. Erb, B.S. (Hygiene), Baltimore, Md.

THE direct method for the enumeration of the bacteria in milk, commonly known as the Breed count has been greatly facilitated in the use by us of a special stain which eliminates many of the time consuming features of the older method, and simultaneously improves the accuracy of the examinations.

In the original Breed method 0.01 c.c. of milk is deposited upon a glass slide, spread over an area of one square centimeter, dried, and then stained by a process requiring separate operations of defatting, fixation and decolorization.

Attempts have been made by others to shorten this technic. Newman has recently published three formulas attempting to combine all the procedures outlined in one operation. We have not found these stains absolutely satisfactory because considerable difficulty was experienced in obtaining completely defatted milk films within a reasonable time.

The following simple technic is submitted therefore for the approval of technical workers in the active milk laboratory. Its simplicity is also an advantage in the academic laboratory. The milk film is prepared as outlined above. The preparations are dried in a warm place upon a level surface and protected from dust. In order to prevent noticeble growth the drying should be accomplished within five to ten minutes, but excessive heat must be avoided or the dry films may crack or peel from the slides during later handling. When dry, the slides are placed in a staining solution having the following composition.

Formula

Ether (sulphuric)	50 c.c.
Methyl Alcohol (absolute)	50 c.c.
Methylene blue (preferably certified)	0.5 gm.

Add the dye to the mixed alcohol and ether. When dissolved filter through paper. The stain is then ready for use. Keep in a tightly stoppered bottle.

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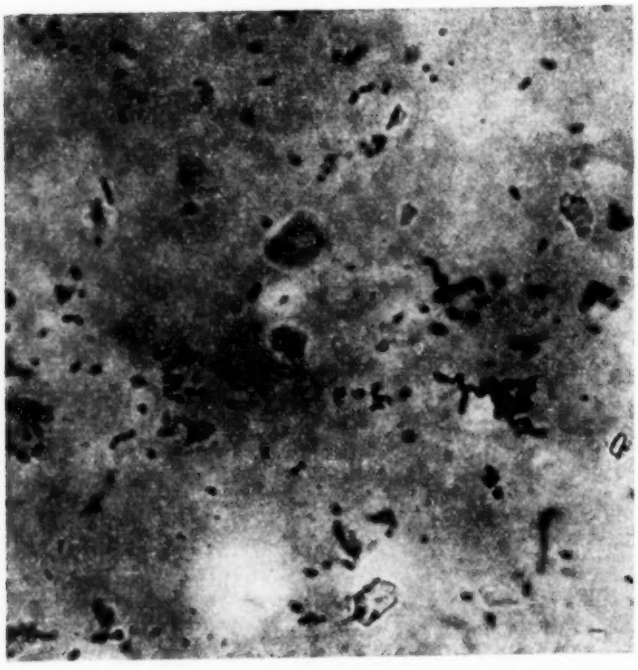

Fig. 1.

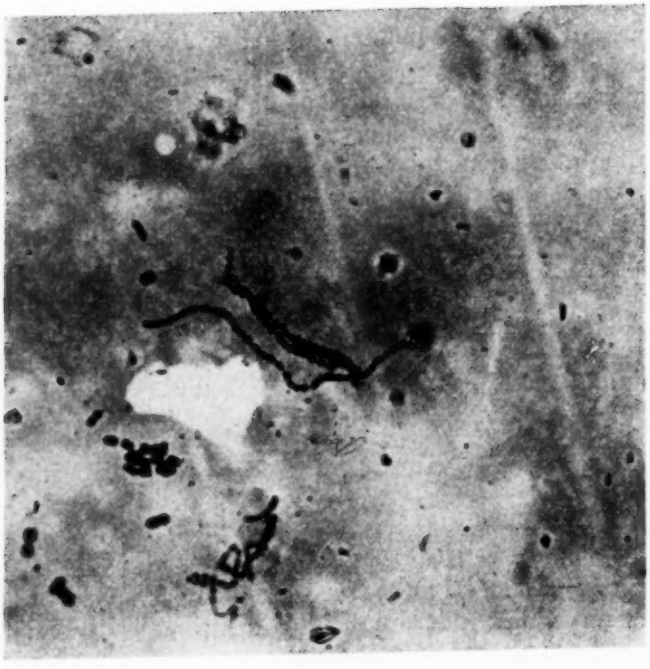

Fig. 2.

The slides are placed in this staining solution for one minute. They are then rinsed gently but thoroughly in water and dried in air. When completely dry, they may be examined with the standardized microscope as described in Standard Methods of Milk Analysis of the American Public Health Association.

The preparations are defatted, fixed, and stained in one operation. There is sharp differentiation between the bacteria and the background. Leucocytes are well stained. The figures illustrate typical microscopic fields stained by this method. Fig. 1 is taken from a microphotograph of a pooled specimen of milk having a high bacterial content. Fig. 2 is from a routine specimen of milk showing a long chain of streptococci.

SUMMARY

1. A one solution stain capable of defatting, fixing, and staining milk films in one minute, has been described.

2. The direct microscopic count of bacteria in milk is greatly facilitated by the use of this stain.

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THE WILHITE STAIN FOR NEGRI BODIES*

By S. W. Bohls, M.D., Austin, Texas

THE following formula for staining negri bodies in rabic brains was developed by Dr. J. T. Wilhite, Director of the Texas State Pasteur Institute of Austin. Since his death in 1927 we have continued to use it with great success.

The formula is as follows:

Methylene Blue	0.9 gm.
Rosanaline Violet	1.5 gm.
Glycerine	125.0 c.c.
Methyl Alcohol	125.0 c.c.

Mix, shake, and let ripen. Make impressions from the Ammon's horn on a glass slide, then take ten drops of the stain with 20 c.c. of 1-50,000 KOH and stain two minutes with heat. Dry and examine under the microscope. The Negri bodies will take the acid stain. Always make fresh stain every morning.

Pasteur Institute.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

HYDROGEN-ION: A Universal Indicator for Hydrogen-Ion Concentration, Bogen, E. J. A. M. A. 89: 199, 1927.

The following mixture has also been developed for this purpose, as it has a range from P_H 1.0 to P_H 10.0, with a continuous change of color with change in hydrogen-ion concentration: phenolphthalein, 100 mg.; methyl red, 200 mg.; dimethylaminoazobenzene, 300 mg.; bromthymol blue, 400 mg.; thymol blue, 500 mg. Dissolve in 500 c.c. of absolute alcohol. Add tenth normal sodium hydroxide solution until the red disappears and the solution becomes yellow (P_H 6.0).

For determining the hydrogen-ion concentration of any solution, add one drop of this indicator to 1 c.c. of the specimen. The colors produced resemble those of the spectrum, thus:

Red	indicates about P_H	2.0	(very strongly acid)
Orange	indicates about P_H	4.0	(strongly acid)
Yellow	indicates about P_H	6.0	(weakly acid)
Green	indicates about P_H	8.0	(weakly alkaline)
Blue	indicates about P_H	10.0	(strongly alkaline)

For closer approximation, a series of standard solutions for comparison can be made with known buffer mixtures as described by Clark and Lubs.

LEPTOSPIROSIS: The Early Diagnosis of Leptospirosis, Schuffner, W. München. med. Wchnschr. 73: 1977, 1926.

Two to 3 c.c. of blood are withdrawn by cardiac puncture from an inoculated guinea pig into a syringe containing citrate solution. The mixture is centrifuged five to six minutes at 1500 r.p.m. Pipette off the plasma and centrifuge it for ten minutes at 1500 r.p.m. The plasma is again transferred to a new tube and centrifuged thirty minutes at 3000 r.p.m. The plasma is decanted and a drop of sediment examined by dark field.

TUBERCULOSIS: Simultaneous Demonstration of Tubercle Bacilli and Elastic Fibres, Jessen, F. Beitr. z. Klin. d. Tuberk. 65: 4, 1926.

When there is plenty of sputum available about 4 c.c. of sputum is mixed with 10 c.c. of sodium hydroxide. With smaller amounts of sputum only 5 c.c. of normal sodium hydroxide is used. This mixture of sputum and sodium hydroxide is heated over a water-bath for twenty minutes at a temperature not higher than 50°, stirring it lightly with a glass rod. It is then centrifuged. A smear of the sediment is allowed to dry, preferably in the incubator, and fixed in a flame. It is stained with carbol fuchsin, heated as usual, and washed; decolorized with 3 per cent alcoholic solution of hydrochloric acid, as usual, and washed, then stained one to two minutes with hematoxylin and cold saturated solution of lithium carbonate, each 1.0; absolute alcohol and distilled water each 20.0 and then washed. They are then decolorized with official iron chloride solution for a few seconds and washed. With this method the tubercle bacilli are stained a beautiful red and greater numbers of them show than with the ordinary method. The author has often demonstrated bacilli by this method in specimens in which they could not be demonstrated by the ordinary methods. The elastic fibers are bluish-gray.

TUBERCULOSIS: Bacteriologic Diagnosis in Infants by Examination of the Stomach Contents, Armand-Delille, P. F. Am. J. Dis. Child. 34: No. 4, 547, 1927.

About 80 c.c. of tepid water is introduced into the stomach and withdrawn by siphoning. This is accomplished by means of a stomach tube which has been lubricated with albolene or olive oil, and which has a tube or funnel attachment. In some cases in which real cavities are found in the lung, purulent sputum is obtained, while in other cases in which bacteriologic examination is particularly important only a few flecks of mucus are seen.

If characteristic sputum is obtained, direct microscopic examination by the Ziehl-Neelsen method is all that is necessary, but in most cases homogenization is required. The liquid is centrifugalized in four tubes and the precipitates united in a porcelain dish to which is added 30 c.c. of water and 10 drops of normal sodium hydroxide; the whole is heated for ten minutes, 50 c.c. of water being added slowly. If the specific gravity is over 1.004, a little alcohol is added. The material is again placed in four tubes and centrifugalized for forty-five minutes, and the precipitate is stained by the Ziehl-Neelsen method. The stomach contents can be kept two or three days before homogenization without destroying the tubercle bacilli. Microscopic examination must be carefully made, because in some cases it is rather difficult to find the bacilli.

The results obtained by this method have been interesting. In sixty-two cases in which only the direct examination without homogenization was used, tubercle bacilli were found in only 10 per cent, while in 110 cases in which the method here detailed was employed 31 per cent were positive.

TUMORS: The Relation Between the Histologic Picture and Prognosis of Tumors, Plaut, A. Arch. Path. & Lab. Med. 3: 240, 1927.

The present possibilities of histologic prognosis are smaller than would appear from the examples given. Conclusions have been drawn from follow-up investigations, but in only a small number of these cases has it been possible to make a histologic prognosis from an excised tumor fragment. Histologic prognosis can be safe only when the tumor is homogenous and when it runs true to course, that means when it does not change its structural and biologic character during the progress of the disease. Carcinoma of the cervix, for instance, fulfils neither of these conditions in a sufficient degree.

General histologic prognosis is feasible in rare instances only; more promising are the endeavors to establish histologic prognosis for special tumors in special locations. The method of clinical statistics is subject to great errors, and only very large figures from homogenous material which are obtained by the same method throughout, can be made the basis for positive conclusions. The many contradictory statements quoted above show that only too well. After extensive studies in such special histologic prognosis, it may be possible in the future to determine which rules for general prognosis will remain; perhaps it will be only those governing the bad prognosis to be made from the highly irregular, pleomorphic structures.

The interpretation of the most used histologic features is so far uncertain. This is true of inflammation in the stroma and in the epithelium itself, maturity of cells and anaplasia and also of the mass relation between epithelium and mesenchyme.

Knowledge of cell division in tumors requires much more study with special reference to the occurrence of amitosis, of intermediate forms of cell divisions and of the different phases of true mitosis. The distribution of the cell divisions may be important also. In order to discuss the term "malignancy," it is necessary to consider separate local extension, metastasis, recurrence, cachexia, local regression during irradiation and occurrence of metastasis after irradiation. Constitutional factors, such as age, race, and sex, must be studied in their interrelations with microscopic pictures.

Histologic prognosis can be a great help to the physician if he is always aware of its limitations. There may be disappointment sooner or later when prognoses are made from microscopic slide examination according to standardized rules. Only the most careful study of individual cases can avail us in the presence of the manifold histologic and clinical phases of malignant tumors.

AMYLOID DISEASE: The Clinical Value of Intravenous Injection of Congo Red in the Diagnosis of Amyloid Disease, Bookman, A., and Rosenthal, J. Am. J. M. Sc. 173: No. 3, 396, 1927.

In a study of 18 cases the authors have found that disappearance of 60 per cent or more of congo red from the blood in one hour is found only in the presence of amyloid disease. A lower rate of disappearance does not preclude the presence of amyloidosis.

One and two-tenths grams of powdered Congo red (Grubler) is mixed with 100 c.c. of water, heated to boiling, filtered through a Berkefeld filter and divided into hard glass ampules each containing about 15 c.c. These are then sealed. The solution must contain no undissolved particles. Ten to 15 c.c. are injected, according to the size of the patient, into a vein at the elbow. The needle is left in place and after four minutes 10 c.c. of the blood are withdrawn. At the end of one hour a second specimen of 10 c.c. is taken.

Serum (which must be free from hemoglobin) is obtained by bleeding through a dry needle into a paraffin tube which is quickly transported, packed in ice, to the laboratory the blood immediately transferred to an unparaffined centrifuge tube and centrifuged at 1500 to 2000 revolutions per minute for at least fifteen minutes. The fibrin is then detached from the walls with a needle, being careful not to approach within a centimeter of the red-cell zone. The clot is allowed to retract and the tube again centrifuged for five minutes. The serum is then pipetted off and the color content of the one-hour specimen determined with any colorimeter, the four-minute specimen being used as a standard. The Kuttner-Leitz Universal microcolorimeter is most useful for this purpose. The addition of dilute hydrochloric acid to the serum changes the color to blue. At times this was found of advantage as a check on the matching of the red color.

CEREBROSPINAL FLUID: Night-Blue Reaction for the Determination of Albumin Increase in Spinal Fluid in Meningitis, Rosenfeld, H. Klin. Wchnschr. 6: 118, 1927.

Two solutions are required for the reaction:

1. A one per mille aqueous solution of night-blue. This solution can be preserved for a long time in a buffer mixture (the hydrogen-ion concentration being 4.6) consisting of 110 c.c. N/10 acetic acid, 90 c.c. of N/10 sodium acetate, 200 c.c. of distilled water. The constancy of the reaction must be controlled from time to time.

The reaction is made as follows: Solution 1 is 10 times diluted with distilled chlorine free water and equal parts of this dilution and of the buffer mixture mixed with each other. Two-tenths c.c. of the cerebrospinal fluid to be examined are put in an acid and alkali-free dry test tube, filling up to 1 c.c. with distilled water. Two c.c. of the night-blue buffer mixture are added. As a control 1 c.c. of distilled water and 2 c.c. of night-blue buffer mixture are put in another test tube. Both tubes are slightly shaken and compared with each other.

In case of meningitic cerebrospinal fluid, rich in albumin, a diffuse turbidity appears immediately. In normal cerebrospinal fluid or one which is rich in globulins (syphilis, tabes, paralysis), however, and in the control tube the mixture remains transparent, blue and clear. Cerebrospinal fluid containing blood also renders a positive reaction because of its marked serum-albumin content.

From a study of 380 cases the author believes this test of distinct value.

TISSUE STAIN: Improved Method of Preparation of Weigert's Elastin Stain, Haynes, F. J. Roy. Micr. Soc. 46: 29, 1926.

To 1 per cent aqueous basic magenta solution add 2 per cent of phenol. Bring to boiling and add an excess of 50 per cent solution of ferric chloride. Boil ten minutes, collect, wash, and dry the precipitate. For use 0.75 gm. is added to 100 c.c. of 96 per cent alcohol containing a few drops of concentrated HCl, boil for fifteen minutes, cool, filter, and add 2 per cent of concentrated HCl. The powder keeps indefinitely and the staining solution two months.

STAINS: The Germicidal Effect of Staining Solutions, Eckfeldt, G. A., and Loser, S. A. Stain Technology. 2: No. 4, 109, 1927.

Gentian violet, crystal violet and carbol fuchsin applied to cover slip preparations for one minute will destroy the majority of nonspore-forming bacteria and yeasts, though they cannot be relied upon to do this consistently and in all cases.

The Gram staining procedure is more effective and nonspore-formers were never found to survive this process.

Methylene blue stains exert very little if any germicidal power and most organisms survived them readily. India ink was totally ineffective.

NEPHRITIS: Diazo Test in Nephritis, Blatner, H., and Fitz, R. J. A. M. A. 88: 985, 1927.

To 1 c.c. of blood plasma or serum is added 2 c.c. of 96 per cent alcohol. The proteins are allowed to precipitate, and the mixture is filtered or centrifugalized. The clear filtrate, or supernatant fluid from the centrifugalized specimen is then collected. To 1 c.c. of this clear fluid is added 0.5 c.c. of alcohol and 0.25 c.c. of freshly prepared diazo reagent. The mixture is boiled for thirty seconds, and then a few drops of a 10 per cent solution of sodium hydroxide are added.

The characteristic reaction is the very rapid development of a pink color, which may disappear rapidly. The intensity of the color and the rate of its disappearance depend upon the concentration of the substance causing the reaction in the blood. The mixture must be watched very carefully during the addition of the alkali, since the pink may appear and disappear within a few seconds. The diazo reagent can be prepared according to the directions of McNee. It consists of a mixture of two solutions, each of which keeps well, but which must always be freshly made immediately prior to a test. The two solutions are prepared in the following fashion:

Solution A: sulphanilic acid, 1 c.c.; concentrated hydrochloric acid, 15 c.c.; distilled water, 1000 c.c.

Solution B: sodium nitrite, 0.5 gm.; distilled water, 100 c.c.

The reagent as used for the test consists of 25 c.c. of Solution A, to which is added 0.75 c.c. of Solution B.

The authors believe a positive reaction to be of ominous significance.

BLOOD CHEMISTRY: A Note on the Preservation of Samples of Blood for Sugar Estimations, Splatt, B. M. J. Australia. 2: 178, 1926.

Confirming the suitability of the method of preservation described by Sanders (5 mg. of 5:1 mixture of sodium fluoride and thymol per 0.2 c.c. of blood).

ASTHMA: A Comparative Study of the Scratch and Intradermal Methods of Skin Testing in Children, Peshkin, A. M., and Fineman, A. H. J. Dis. Child. 34: No. 5, 815, 1927.

Ninety-one children with asthma and nine with eczema, urticaria or angioneurotic edema, ranging in age from eight months to fourteen years, were tested by the scratch and intradermal methods with thirty-three corresponding proteins. Dry powdered extracts (Wodehouse) were employed for the scratch and routine fluid extracts (Coca) for the intradermal tests. The fluid extracts were also applied with the scratch method when the intradermal test gave a reaction above the control. All of these children were under observation for at least one year.

In interpreting the reactions obtained with the scratch method, all reactions above the control were considered positive. With the intradermal reaction, although all reactions above the control were recorded, only reactions of two-plus or more were considered definitely

positive. This interpretation is in accordance with accepted standards for this method. However, it appears that one-plus reactions should not be entirely ignored in spite of the fact that they are most frequently nonspecific.

Thirty-eight children of the entire series reacted negatively with the scratch method. With the intradermal method, fifteen children gave negative reactions, and in twenty-three the reactions obtained were plus minus and one-plus only.

Sixty-two children reacted positively to the scratch method. With the intradermal method, twenty-six patients gave reactions not larger than one-plus and in thirty-six two- and three-plus.

No patients were encountered who gave definitely positive reactions with the intradermal method and negative reactions with the scratch method.

The total number of negative reactions obtained with the scratch method were 3,032. The corresponding reactions with the intradermal method were definitely positive (++ and +++), in 0.53 per cent; one-plus, in 4.15 per cent, and negative (− and ±), in 95.32 per cent.

The total number of positive reactions (± to ++++) obtained with the scratch method was 233. The corresponding reactions with the intradermal method were definitely positive in 19.31 per cent, one-plus in 27.47 per cent and negative in 53.22 per cent.

Pseudoreactions or nonspecific reactions were common with the intradermal method. Practically all the plus minus and a majority of the one-plus reactions were of this character. The nonspecific reactions occurred most commonly with the following substances in their order of frequency; house dust, chicken meat, rice, wheat, chicken epithelium, tobacco, corn, pyrethrum (insecticide), goose and goat epithelium and lamb. Pseudoreactions with the scratch method were relatively infrequent.

Four children gave a history of positive pollen reactions and negative ragweed reactions with both methods of testing (the fluid extract for the intradermal test was employed up to a concentration of 0.25 mg. of nitrogen per cubic centimeter); they gave positive reactions by the conjunctival test to the dry powder. The latter method testing has been shown by Peshkin to be a rapid, safe and reliable method of demonstrating sensitiveness to pollen in patients refractory to skin tests.

General reactions occurred in 2 per cent of the patients tested with the intradermal method and none with the scratch method.

The scratch test is superior to the intradermal test for the following substances: rabbit hair, horse dander, ragweed, cottonseed, duck and goose feathers, cat and dog hair, mustard, and egg-white.

The intradermal test is superior to the scratch test for the following substances: house dust, chicken epithelium, chicken meat, wheat and corn. In the presence of negative scratch reactions to horse serum and goat hair, the intradermal method, in a small percentage of the cases will reveal definitely positive reactions.

From the standpoint of safety and the determination of etiologic sensitizations, the scratch method would appear to be superior as a routine procedure for testing. The value of the intradermal method cannot be ignored. One method should not be employed to the exclusion of the other.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Daily Consultations. Les Consultations Journalieres**

A SERIES of small handy reference volumes on subjects of interest to the general man and to those interested in the special lines to which each volume is devoted. Their special interest to the American students lies in their exposition of the French viewpoint on the subjects discussed. Radiotherapy including x-ray and radium treatment and ultra-violet ray therapy is covered by W. Vignal, electro radiologist of the hospitals of Paris. This is a volume of 425 pages in which therapy is discussed in the deepest detail, from its beginnings. Little attention is given to diagnostic roentgenology.

The volume on gynecology is contributed by E. Douay, Chief of Gynecology at the Broca Hospital. This volume is decidedly more monographic than the former as it deals with four selected phases of gynecology. These are menorrhagia and metrorrhagia, considered as one, leucorrhea, dysmenorrhea, and sterility. The first three as the author points out are the three great symptoms that dominate all gynecology. The discussion of all four subjects is comprehensive and many valuable therapeutic points are to be found in the volume.

A rather large volume on diseases of the esophagus, stomach, and duodenum by Maurice Delort, and on diseases of the oral cavity including the teeth by F. Nidergang, both of Paris, forms an excellent reference manual. The illustrations, particularly the radiograms, are well reproduced.

An index of therapy entitled Les Formules Usuelles by Segard and Laemmer takes up the various commoner diseases in symptomatic and pathologic classification using an alphabetic order. Under each subject the outline for therapy, including many special prescriptions, is presented in tabular form.

No one man will have need for all of the volumes but the internist or general practitioner who has an acquaintance with French will find much of value in the last three volumes mentioned.

Les Indications Cliniques de L'Electro-Radiotherapie †

THIS is a new idea. The author includes electrotherapy, diathermy, radiotheromotherapy, phototherapy, actinotherapy, x-ray, and radium treatment and in the discussion of each clinical condition designates which of these methods of treatment or what combination should be utilized. Technic is included. Statistical results are not included.

*Daily Consultations. Les Consultations Journalieres. G. Doin & Co., Paris, 1927.

†Les Indications Cliniques de L'Electro-Radiotherapie by Etienne Piot. Paris, Librairie Octave Doin, Gaston Doin & Cie, Editeurs, 8, Place de L'Odeon, 8. 1927.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*The Clinical Interpretation of Blood Chemistry**

IT IS not essential that the general practitioner or the clinician be adept in the manipulative technicalities of blood chemistry studies. He should, however, be thoroughly familiar with the significance and clinical interpretation of the findings. Unfortunately the latest information on the clinical significance is not always readily available since the reports often appear in highly specialized journals and are widely scattered through the literature. The author has collected the necessary information in this small volume. It is not a laboratory manual and gives little detail of laboratory procedure but starts the study of each blood chemical determination at the time when, the laboratory work being completed, the final report has been made.

In addition the author includes a large variety of food tables which are thus available for handy reference, and a general outline for the management of those diseases in which the blood chemistry shows alterations.

Self-Care for the Diabetic*

Self-Care for the Diabetic†

A MANUAL for the use of diabetic patients which follows the same general line as the several similar volumes which have been developed in this country and should be of interest to us in the United States as illustrating the British attitude toward the treatment of this highly important subject. The reviewer feels that the volume has one shortcoming in that it does not provide ample facility for the patient to calculate his own diet and to provide himself with a wide variety of foods while following his dietary prescription.

The Carrier Problem‡

IN THE realm of the infectious diseases the carrier problem is as true and as pressing a problem as any that exists. And yet surprisingly few have made any extended special studies in this field. The last monograph on this subject appearing in England prior to the one under review appeared as long as sixteen years ago. The present author makes no claim to inclusiveness in his discussion of the subject but presents the outstanding facts of the carrier stage as we know it today. The work covers the enteric infections, diphtheria, meningococcus infection, pneumococcus and streptococcus infections, poliomyelitis, bacillary dysentery, cholera, protozoan infections, and a small group of miscellaneous diseases. Under each he describes the probable development of the carrier state and discusses those methods which we have at present at our disposal for the prevention and relief of this condition. His conclusion is that at present we know distressingly little concerning the mode of development of the carrier state and even less of its cure.

And yet in epidemiology the subject is of utmost importance for were we able to recognize and cure the carrier these diseases would stand in good way of being eliminated. Yellow fever and malaria neither of which the author discusses, for he is dealing primarily with human carriers, are examples of the great potentialities in the hands of preventive medicine provided a satisfactory method of eliminating the carrier is found.

The carrier problem is not alone for the epidemiologist. Few states call for as closely coordinated study by a wide variety of interests. The bacteriologist, the immunologist, the protozoologist and the public health official must all cooperate and for greatest success the family physician whose contact with the individual is the most direct should cooperate as thoroughly.

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*The Clinical Interpretation of Blood Chemistry. By Robert A. Kilduffe, A.B., A.M., M.D. Director, Laboratories, Atlantic City Hospital; Consulting Serologist, Betty Bacharach Home for Children, Serologist, Jewish Seaside Home, City Bacteriologist, Atlantic City, Formerly Director, Laboratories Pittsburgh Hospital, Pittsburg, Pa., Cloth. Pages 186. Lea & Febiger, Philadelphia, 1927.

†Self-Care for the Diabetic. For the Use of Diabetic Patients. By J. J. Conybeare, M.C., M.D. Oxon., F.R.C.P. (Lond.), Assistant Physician to Guy's Hospital. Pages 70. Cloth. Oxford University Press. American Branch, N. Y., 1926.

‡The Carrier Problem. By K. C. Paul, M.B., B.S., Sometime Research Student in Medicine, University of Madras. With foreword by David Nabarro, M.D., F.R.C.P. (Lond.), Director of the Research and Pathological Laboratories, The Hospital for Sick Children. Pages 102. Cloth. Oxford University Press American Branch, N. Y., 1926.

While vast sums are being spent yearly on endowments for medical research the reviewer would suggest the establishment of an institute primarily for the study of the carrier state, an institute which might with benefit adopt certain features of the Framingham tuberculosis demonstration and of the James Mackenzie Institute. In other words it would be located in a relatively isolated, unchanging community, be provided with the best of laboratory facilities, and would elicit the hearty interest and cooperation of the practicing physicians in the community. For best results the study of the carrier problem will necessarily require concomitant laboratory investigations and clinical application directed to the individual with contemporary epidemiologic supervision applied to the group or community.

The Enlarged Prostate *

A VOLUME devoted primarily for the urologic surgeon. While the usual method of treatment remains prostatectomy, either suprapubic or perineal, the author reports a number of instances of successful results by partial removal by means of per-urethral operation, usually with diathermy. He also mentions the good results that have been obtained particularly in Germany following x-ray treatment. He remarks that previous x-ray makes subsequent operation no more difficult but that it appears in some cases to delay healing of the abdominal wound.

The volume makes a quite complete work on the prostate gland, taking up as it does the anatomy and physiology of the prostate, the pathology of enlargement, symptoms, diagnosis and treatment of hypertrophy.

Prostatectomy is at no time a simple or easy procedure. It carries with it a definite mortality. Walker quotes a large series of records in which he shows that skill decidedly lessens operative mortality. The death rate in clinics devoted entirely to genitourinary surgery runs between one-third to one-half the death rate in otherwise equally efficient and well recognized general hospitals. At St. Thomas' Hospital, London, the mortality was 20.3 per cent, at St. Bartholomew's 22 per cent, while at St. Peter's, a special hospital devoted entirely to the treatment of genitourinary diseases the death rate was 9.28 per cent.

The Treatment of Chronic Arthritis and Rheumatism †

A RATIONAL presentation of our present understanding of the problem of arthritis and allied diseases. The authors have no pet theory nor an unusual method of treatment. They present various therapeutic measures more generally used, for what they are worth. The authors do stress the importance of recognizing the various types of joint disease and instituting the treatment appropriate for the particular type. The differentiation of types and sub-varieties is well worth the reading by all who are interested in this subject. A chapter on common mistakes in diagnosis emphasizes the importance of ruling out other types of pathology such as static deformities, newgrowth, diseases of the central nervous system, certain diseases of bones and joints, and referred pain due to visceral disease.

Neuritis and Neuralgia*

Neuritis and Neuralgia ‡

AN EXHAUSTIVE and informative treatise on disease of the peripheral nerves and neuralgia of central origin such as migraine, which will be of especial value to the neurologist and the neural surgeon.

*The Enlarged Prostate. By Kenneth M. Walker, F.R.C.S., M.A., M.B., B.C., Jacksonian Prizeman and Hunterian Professor, Royal College of Surgeons, 1911, 1922, and 1924. Lecturer in Venereal Diseases, St. Bartholomew's Hospital, Surgeon with charge of Genito-Urinary Department, Royal Northern Hospital, Surgeon to St. Paul's Hospital. Cloth. Illustrated. Pages 193. Oxford University Press. American Branch, New York, 1926.

†The Treatment of Chronic Arthritis and Rheumatism. By H. Warren Crowe, D.M., B.Ch. (Oxon.), M.R.C.S., L.R.C.P. Cloth. Illustrated. Pages 187. Oxford University Press, American Branch, N. Y., 1927.

‡Neuritis and Neuralgia. By Wilfred Harris, M.D. Cantab., F.R.C.P. Lond., Senior Physician to St. Mary's Hospital, and to the Hospital for Epilepsy and Paralysis, Maida Vale. Cloth. 418 pages. Illustrated. Oxford University Press. American Branch, New York.

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EDITORIALS

The Statistical Study of Diabetes

KNOWLEDGE, in the last analysis, depends upon the accumulation, digestion, and interpretation of experience and especially is this true in the development of diagnostic and therapeutic acumen. For this reason the careful survey of any large series of observations is of great value to the physician at large, and such a survey of diabetes, embracing two thousand cases, has recently been published by John[1]

Despite the volume of work which has been done the benefit which has accrued to the diabetic in comfort, in the avoidance of complications, and in the prolongation of life, there is still much to be learned and much to be done before any diabetic in any place may be assured of competent and intelligent handling.

The use of insulin, for example, is now widespread but it may be questioned if a thorough knowledge of the minutia of insulin treatment essential to therapeutic success is equally widespread.

John's report contains much that is of interest to the physician at large and it is, for that reason, herewith summarized.

The incidence of diabetes among all diseases seen during the course of this study (87,449 cases from 1921 to 1927) was 2.28 per cent. The incidence as to sex was males 46.75 per cent and females 53.25 per cent. The age incidence was highest in the fourth decade, although most of the cases came from the fifth, sixth, and seventh decades.

There has been much speculation as to the importance of various factors in the etiology of diabetes. As John comments, the fact that obesity usually first manifests itself in the decade in which the incidence of diabetes is greatest, the fourth, forces some thought as to the exact relation one bears to the other. Infection, also, has been regarded by some as a predisposing factor of greater or less importance. In the present series, however, as infection predominates in the first two decades in which the incidence of diabetes is low, its marked importance as an etiologic factor may be doubted in John's opinion.

There was a hereditary history of diabetes in 5.3 per cent and a familial history in 4.5 per cent, figures somewhat lower than those usually encountered in the literature.

The highest blood sugar noted was 900 mg. per cent.

The observations as to the correlation between glycosuria and hyperglycemia are of interest to the laboratory worker who must explain at times that the absence of the expected correlation does not necessarily predicate technical error in the laboratory.

In 159 cases in which glycosuria was present the blood sugar was below 180 mg., and in 26 cases with glycosuria, the blood sugar was only 120 mg. per cent. In one case, on the other hand, there was no glycosuria with a blood-sugar level of 390 mg. per cent, and others, though not so striking which illustrate how relatively impermeable the renal filter is to dextrose.

As John says: "The point which one must derive from these data is that the blood-sugar level does not by any means disclose the level of the renal threshold.

"The urine collected at the moment when the blood is taken for sugar estimation is not the urine which has been secreted during those particular few minutes, but rather includes that urine plus all the urine which was secreted during the period of time since the last preceding voiding. It is necessary, therefore, to determine what the blood-sugar level was throughout that period. Did it reach a high level above that of the renal threshold, and gradually drop to the level at the time the specimen was taken, or was it at the same level at which it was found throughout the period since the last preceding voiding? The figures per se do not give definite information as to the patient's renal threshold. . . . It is interesting, however, to observe that in a good many definitely diabetic patients in whom the blood-sugar content is low glycosuria is present."

John presents some very pertinent observations upon insulin treatment. He says: "In the treatment of persons with diabetes there are four chief

objectives: (1) To get the patient as nearly normal as possible; (2) to find out what is required to gain and maintain the normal state; (3) to plan a livable, not a starvation, diet so that the patient may hold his job; and (4) to instruct the patient in the routine he must follow if he is to maintain the best possible status."

Between simple starvation with all the consequences it entails besides lowering the blood sugar, and a livable diet from the start with enough insulin to lower the blood sugar to normal as desired, John favors the latter method as the more economically logical and more conducive to the general well-being of the patient. And he emphasizes that it is not the diet but the insulin which must be juggled to accomplish this end. Inasmuch as, he says, insulin loses its effect relatively when the dosage is increased, one should employ the smallest amount which will accomplish the desired result.

Those who fear that once begun insulin must be used forever will find comfort in the repetition of the fact, emphasized anew by John's series, that this belief is a fallacy.

The observations of John concerning the treatment of coma are of practical interest.

He comments upon the common belief that when a diabetic is unable, for one reason or another, to take food, starts vomiting, and enters the path leading to acidosis, it seems logical to withhold insulin for fear of hypoglycemia, thus increasing the likelihood of coma. The physician, however, must forcibly impress upon the patient and his attendants the fact that the body continues to burn calories just the same, the only difference being that when food is taken the calories come from without whereas they are supplied by the burning of the body tissues in the absence of food. In either case insulin is required to accomplish the combustion so that vomiting is not a sign for the cessation of insulin but an evidence of the need for insulin.

In the treatment of coma John believes that three points should be stressed:

"1. The administration of the sufficient amount of insulin to produce the desired effect of clearing up acidosis and lowering the blood-sugar level; this may take anywhere from 80 to 700 units in twenty-four hours.

"2. The use of 10 per cent dextrose intravenously.

"3. Hospitalization of the patient."

In the treatment of coma there has been some difference of opinion as to the advisability of administering glucose intravenously with insulin because, as has been said, this simply increases the hyperglycemia. But, as John points out, if the amount of insulin is calculated in accordance with the total amount of glucose in the blood, not only will there be a fall rather than a rise,[2] but in the combustion of the total glucose the acetone bodies will be consumed, and experience has shown that large and frequent doses of insulin will be absorbed by the patient without difficulty.

New data are brought by John illustrating what indeed requires appreciation: the variability of individual response to insulin and the necessity

in some cases for what he calls "the wearing-out process" meaning the strict adherence to routine in cases showing a slow uneven progress, but in whom eventually a normal state may be established and, once obtained, maintained by a remarkably small amount of insulin.

John calls attention to the brilliant results now possible in the surgery of diabetes by careful pre- and postoperative treatment with insulin and glucose intravenously.

The paper above thus briefly summarized constitutes a contribution to the subject of diabetes which well repays study in the original.

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—*R. A. K.*

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